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Award Number:
W81XWH-07-1-0201

TITLE:
"Determination of Optimum Vitamin D Nutrition in Young Women"

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CONTRACTING ORGANIZATION:
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REPORT DATE:
October 2012

TYPE OF REPORT:
Revised Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: (Check one)

☒ Approved for public release; distribution unlimited

☐ Distribution limited to U.S. Government agencies only;
report contains proprietary information

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
October 2012

2. REPORT TYPE
Revised Final

3. DATES COVERED
30 September 2007- 29 September 2012

4. TITLE AND SUBTITLE
Determination of Optimum Vitamin D Nutrition in Young Women.

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-07-0201

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. J.C. Gallagher, M.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Creighton University

Omaha NE 68178-0410

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The main objective of this proposal was to study the effect of increasing doses of vitamin D3 in a group of young Caucasian and African American women with vitamin D insufficiency, that is with a serum 25OHD < 20 ng/ml) and an adequate calcium intake of 1200 -1400mg/day. African American women generally have lower levels 25-vitamin D.This was a double blind randomized placebo controlled study. There were 5 treatment arms, four vitamins D3 dose groups - 400, 800, 1600, 2400 IU/day and a placebo). Calcium citrate tablets were given to maintain the calcium intake between 1200-1400mg/d. The study plan was to recruit up to 100 Caucasian and 100 African America women subjects between ages 25 to 45 years. The primary outcomes were the changes in serum 25-hydroxyvitamin D and serum Parathyroid hormone. Secondary outcomes were calcium absorption and for safety measurements of serum calcium and 24 hour urine calcium. 198 women(119 Caucasian,78 African American)were randomized and 128 completed the study one year later. The final results show the response to oral vitamin D is the same in both Caucasian and African American young women. The implication is that vitamin D metabolism is the same and that lower vitamins D levels in African Americans are due to increased skin melanin filtering out the ultraviolet light. This is the first study to estimate the Recommended Dietary Allowance (RDA) in young people and shows that the RDA for vitamin D is400-800 IU daily.

15. SUBJECT TERMS- none provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU		**19b. TELEPHONE NUMBER** *(include area code)*

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FINAL Report
(Oct 2009-Sept 2010)

Grant/Cooperative Agreement Number:
Proposal No. PR065013, Award No.W81XWH-07-1-0201, HRPO Log No. A-14205

Grant/Cooperative Agreement Title:
Protocol, "Determination of Optimum Vitamin D Nutrition in Young Women"
Acronym ViTADAS study. Vitamin D in young Adult Subjects

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Abstract

The main objective of this proposal is to study the effect of increasing doses of vitamin D3 in a group of young (age 25-45 years) Caucasian and African American women with vitamin D insufficiency, that is serum 25-hydroxyvitamin D < 20 ng/ml. An adequate calcium intake of 1200 -1400mg/day was maintained with calcium supplements after determining the usual calcium intake from a 7-day food diary. The study design is a double blind randomized and placebo controlled. There are 5 treatment arms, four vitamin D3 dose groups - 400, 800, 1600, 2400 IU(International Units) per day, and a matching placebo. Calcium citrate tablets are given to maintain the calcium intake between 1200-1400mg/d. Although the sample size estimation was for 40 per dose group, upto 50 per group could be randomized to allow for excessive dropouts. The primary outcomes are changes in serum 25-hydroxyvitamin D (25OHD) and

serum parathyroid hormone (PTH). Major secondary outcomes are calcium absorption and evaluation for safety using serum calcium, creatinine and 24 hour urine calcium.
DRI's (Dietary Reference Intakes or) or vitamin D have *never* been determined for this age group and this is a gap in our knowledge. The results from this study will provide information helpful for making recommendations on the Recommended Dietary Intake (RDA) for young people.

This study was designed to estimate the RDA (Recommended Dietary Intake) for vitamin D in young people. The background to this proposal is based on the following information. Dietary Reference Intakes (DRI) are reference intakes for the healthy population for long term intake. The DRI's are recommended by the Food and Nutrition Board that is situated in the Institute of Medicine (IOM). The last recommendations were made in 1997 but the Committee was reconvened in 2009-2010 to update their recommendations. Below is the background to the IOM

Part of science standard for federal nutrition guidance
HHS/USDA [Department of Health and Human Services / U.S. Department of Agriculture (*Dietary Guidelines for America)*
USDA *MyPyramid*
Health Canada *Canadian Food Guide*

Statutory or *De Facto* standard for virtually all food program
4 official USDA food plans
Food stamp allotments/WIC (Women Infants and Children.) food packages
DOD service member
Standard of comparison for USDA review of food distribution programs on Indian Reservations

Our DOD supported study was designed to add information on the Recommended Dietary Intake (RDA) for young people since there was no published data. For clarification the key definitions used in the DRI's are as follows:

EAR (Estimated Average Requirement) -- the median requirement that covers the needs for about 50% of the population for the nutrient for each age-sex group
Recommended Dietary Allowance (RDA) intake covers needs of 97.5% of the population for each age-sex group
Tolerable Upper Level (TUL) this is an intake (food and supplements) above which risk begins to increase

To estimate the DRI's the IOM reviews all of the data that supports these recommendations. In recent years the measurement of vitamin D in the blood, specifically serum 25-hydroxyvitamin D (25OHD) has become a biomarker of vitamin D exposure. Vitamin D comes from diet usually fish or supplemented foods particularly dairy products, but natural sources of vitamin D are low. More vitamin D in the blood may come from sunlight that acts on skin to form vitamin D. Levels of vitamin D increase in summer and decrease in winter. Sunscreen blocks the effect of sunlight on the skin and lowers vitamin D levels in the blood.

The diagnosis of vitamin D insufficiency measured as serum 25OHD has become more common in the last 3 years as health professionals became more aware of this issue. In 2002 the World Health Organization (WHO) suggested that a serum 25OHD level of <20 ng/ml was sufficient for health (1). An Endocrine society guidelines in 2011 suggested that a serum 25OHD level of >30ng/ml is optimal for health (2). In contrast the Institute of Medicine (IOM) in 2011 after its review of the literature said that the evidence only supported a serum 25OHD level > 20ng/ml and this evidence was based on bone health and not other diseases (3,4). The different recommendation between these two opinions has an unusually large impact for two reasons. Firstly, studies such as NHANES show that 30 percent of the population in North America have serum 25OHD levels below 20ng/ml and as much as 50 percent have values below 30ng/ml (5),

Thus, testing the blood for vitamin d insufficiency has a large economic impact, perhaps as much as 20 billion dollars has been spent in the last 2 years on measuring serum 25OHD because health professionals were concerned that their patients were not meeting the 30 ng/ml level.

Secondly the amount of vitamin D needed (RDA see definition above) to reach a level of 20 ng/ml is much less than reaching a target of 30ng/ml. The IOM did not think there was an evidence basis for recommending a target serum 25OHD level of 30ng/ml considering that these recommendations are for long term healthy populations. In 2011 the IOM published new DRI's for vitamin D but it should be noted that there was no data on young people and none on ethnic groups other than Caucasians.

	EAR (IU/day)	RDA (IU/day)
1-70 years	400	600
>70 years	400	800

This grant application was written in 2004 because there were no systematic dose response studies on vitamin D in young people and so it was impossible to determine the RDA for vitamin D. In 1997 the IOM recommended an RDA of 600 IU/day in young people, and in 2011 the IOM did not change its recommendations because there was no new data. This study will be the first dose response study of vitamin D in young people.

In 2004 when this application was written we used a target serum 25OHD level of 30ng/ml on which to base our hypothesis. We postulated that the dose of vitamin D to meet an RDA based on the 30ng/ml figure would exceed 1700 IU/day in Caucasian and 2000 IU/day in African American women. This is much higher than the 1997 recommendations.

Our study plan was to measure the dose response of vitamin D3 400, 800, 1600, and 2400 IU/day compared to a placebo group on serum 25OHD.

Body

Timeline. Funding for this study started on October 6, 2007. The first six months involved development of a protocol, construction of subject charts, submission to the local IRB and approval by DOD. There was a significant delay in obtaining final approval by HRPO.

10/10/2006 Development of protocol and forms
12/13/2006 Initiate document submission Amber Stanley
1/25/2007 Protocol submitted to DOD Dr. Gallagher
9/8/2007 IRB approval of protocol Dr Gallagher
10/1/2007 Funding started
10/16/2007 PEF comment Johann Kidwell
11/19/2007 Reply to PEF. Dr. Gallagher
12/20/2007 PEF further comments. Johann Kidwell
1/10/2008 PEF further comments. JohannaKidwell
1/24/2008 Creighton IRB approval of protocol & forms. Dr Gallagher
2/18/2008 UNMC IRB approval. Dr Gallagher
2/19/2008 Study drug arrived.
2/26/2008 DSMB Conference completed. No issues arose.
3/19/2008 Final approval by HRPO.
3/19/2008 Clinical trial registered NCT00662844.
4/1/2008 Recruitment started.
9/29/2010 Recruitment finished
8/24/2011 Last subject completed study

Statement of Work

Task 1: To examine the dose response effect of vitamin D_3, 400, 800, 1600 and 2400 IU /d on serum 25-hydroxyvitamin D (25OHD) and parathyroid hormone (PTH) levels in young women with vitamin D insufficiency (serum 25OHD <20 ng/ml) in the presence of an adequate calcium intake in winter.

Task 2: To establish a dose of vitamin D_3 which will increase serum 25OHD above 30 ng/ml in 97% of study subjects in winter and reduce serum PTH to the normal mean levels.

Task 3: (i) To study the dose response effect of vitamin D_3 on calcium absorption
(ii) To study the effect of vitamin D_3 urine bone resorption markers and bone mineral density (BMD).

Task 4: To compare the effects in Caucasian and African American women.

Task 5: To establish the safety of these doses relating to hypercalcemia/ hypercalciuria.

Some of these tasks overlap since Task 4 is built into the study design of Task 1,2 & 3

RESULTS:

TASK 1,2 4 The study details of tasks 1,2 are comprehensively described in the following pages in the following order:

This task is completed

Study design
Sample size calculations
Randomization
Statistical analysis
Results

Study Design
This study is a 1 year double blind, randomized prospective clinical trial aimed at establishing the dose of vitamin D3 required to reach a serum 25OHD level of 30 ng/ml and normalize serum PTH in the study subjects in winter in young Caucasian and African American women with a starting serum 25OHD level of <= 20 ng/ml and sufficient intake of calcium.

Study Groups
200 adult women were to be randomly assigned to the following groups.
1. Placebo group (control)
2. 400 IU vitamin D3
3. 800 IU vitamin D3
4. 1600 IU vitamin D3
5. 2400 IU vitamin D3

Sample size justification: The primary endpoints for sample size calculation were serum 25OHD and PTH at the 12-month visit. With serum 25OHD as the outcome, we used our previous studies to estimate a 2.3 standard deviation (SD) difference between the dose groups. With serum PTH as the outcome, the placebo group was expected to have a mean final serum PTH of 34.6 ng/ml and the 2400 IU dose group is expected to have a mean final serum of 26.3 ng/ml with the standard deviation for the entire population estimated to be 9.8. Assuming that the means for the dose groups are approximately equally spaced in this range, a 0.05 level of significance, and a total drop-out rate of 10 percent; 20 subjects randomized to each dose group for Caucasians and African Americans will provide over 90 percent power to detect a 2.3 SD difference between the doses for serum 25OHD as well as a 0.84 SD difference between doses for serum PTH. This will also provide 80% power to detect a 0.84 SD difference between the race groups, assuming a clinically significant difference between the races to be 9 ng/ml (SD=10.7) for serum 25OHD and over 80% power to detect a 0.20 SD difference between the race groups for PTH, assuming a clinically significant difference in PTH between the races to be 2 ng/ml (SD=9.8).

Randomization: African American and Caucasian women who met the eligibility criteria were randomly assigned to one of five dose groups. Persons blinded in the study were the subjects and

the study personnel including the principle investigator; the statistician had access to the treatment assignments. The randomization method was randomized blocks with block sizes of 4 and 8, stratified by screening serum 25OHD level (<15 vs. >=15 ng/ml for Caucasians and <12 vs. >=12 ng/ml for African Americans). These levels were selected because BMI is associated with lower serum 25OHD levels and this could avoid unbalancing of the groups.The study statistician generated the randomization list with SAS software.

Statistical Analysis: Analysis included all subjects that were randomized. For subjects who dropped out or were removed from the study their data was included in the analysis if it was available. Subject characteristics at baseline were descriptively compared between the dose groups with data presented as means and standard deviations and counts and frequencies.

Mixed effects models were used to estimate dose response curves for serum 25OHD and PTH. Dose (as continuous) and time (as categorical, baseline, 6 and 12 months) were included as fixed effects and subject and study site were included as random effects. Quadratic terms were explored for dose as well as log transformations as well as interactions. Unstructured covariance matrix was chosen. Model fit was examined by looking at various residual plots. 1000 bootstrapped samples were used to determine the 95% prediction limits for the 12-month serum 25OHD levels. The dose at which subjects reach the RDA is the dose at which the 95% prediction lower limit is above 30 ng/ml serum 25OHD. The estimate of the average requirement (EAR), the dose at which serum 25OHD is > 30 ng/ml in 50% of the subjects will be found similarly.

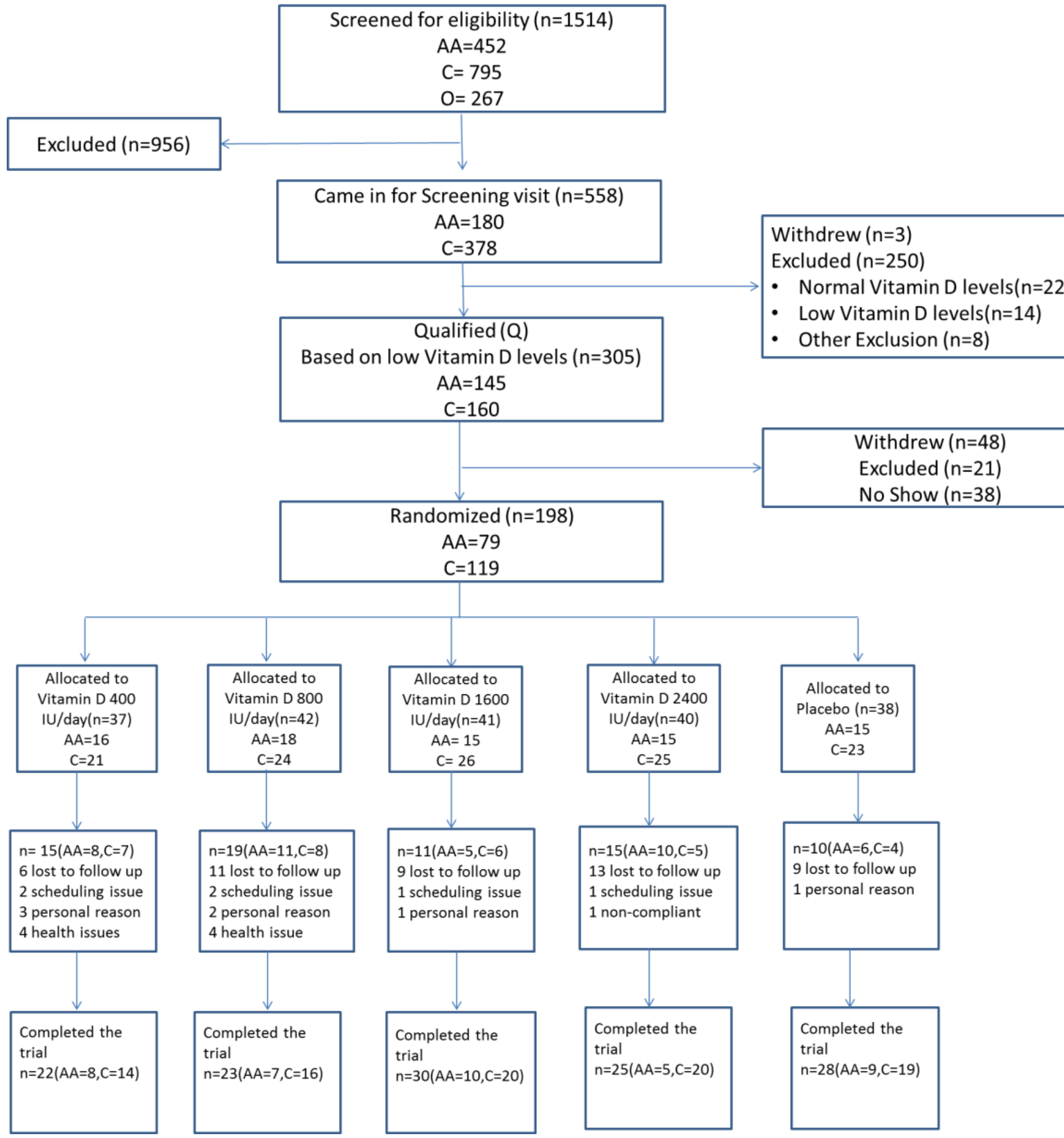
Missing serum 25OHD and PTH caused by loss of follow-up are possibly related to the subject's dose (non-ignorable missingness). Within the mixed effects models we tested for missing completely at random (MCAR) following the method of Park and Lee (6). Briefly, this method uses indicator variables for the missing data pattern and the coefficients for these indicator variables are tested to determine which are significantly different from zero. The missing data mechanism should not be assumed to be MCAR if any of these indicator variables are significantly different from zero.
The mixed model method was used to fit a generalized linear mixture model that is appropriate when the MCAR assumption is violated, to examine factors associated with outcome (7). In this approach, the change in outcome over time is assumed to be dependent on the dropout time. Multivariate mixed effects models were also examined. The models adjust for known covariates based on clinical experience: season at randomization, age, BMI category (<25, 25-29.9, >=30), calcium intake, smoking status, alcohol use, and serum creatinine. Interactions between dose and covariates were explored and interactions significant at the 0.10 level were retained in the model. SAS software (SAS Institute Inc., Cary, NC) was used for the statistical analysis. R version 2.11.0 was used to create graphical displays. P-values less than 0.05 are considered statistically significant.

Results

CONSORT TABLE (figure 1) Recruitment and disposition of subjects -
Summarizes the flow of subjects
Problems encountered in the study with recruitment and follow up
Reasons for withdrawal

Figure 1
Consort diagram

Baseline characteristics



Recruitment:

Because serum 25OHD is lowest in the months January to May the window for recruitment was restricted. As a result of the delayed approval by HRPO we were only able to recruit for only 2 months in the first year- 2008.

Summarizing our activity we screened a total of 1514 women on the phone. 590 qualified by phone screening and scheduled an informational screening visit came in and signed consent forms. In the next stage 558 came for a screening visit consent and had blood drawn for vitamin D measurement. 305 qualified on the basis of low serum 25OHD.In the next visit 198 were randomized to a treatment group. A complete summary to date of our subject contact and recruitment is shown in Figure 1 as a CONSORT diagram. It illustrates some of the problems

and difficulties associated with recruitment. Of those subjects that had a screening blood sample drawn, 55% qualify on the basis of low serum 25OHD; however, only 35% of those screened would eventually be randomized to study drug. Another issue is that 27% of those that schedule an informational screening meeting are ‘no shows’ and many of them are repeated ‘no shows’ even after rescheduling. The same can be seen of those subjects that qualify for randomization. 28% of these subjects do not get randomized to treatment (‘no shows’ or ‘withdrew’). In the ‘no show’ category are those subjects who we have not been able to contact to date and have not returned phone messages. If any ‘no show’ has been contacted they may have come back or given us a reason why they are declining participation (‘withdrew’) and then have been moved out of the ‘no show’ category.

We have employed visit reminder post cards and phone calls/messages as methods to help alleviate the ‘no show’ issue. African American subjects have been difficult to recruit and during year two we took steps to aid in recruitment. Recruitment flyer mailings, targeted mailings, multiple radio advertisements, recruiting booths at health events and recruitment informational events at companies have all been employed. While continuing the above in year three we also added walking campaigns targeting small businesses in several areas of town, leaving flyers with business owners that allow community event postings in their stores; as well as meeting with church and community/business groups. Our goal has been to try and get a one-on-one informational session with a potential subject in order to make them feel comfortable with the project and staff.

Randomization Goals

The target randomization of 100 has been met for the Caucasian subjects, with a total of 119 eligible subjects enrolled. A total of 79 African American subjects have been enrolled, the randomization goal was not met. A total of 198 subjects were randomized on the study

Completion of Study Visits

There are 5 total study visits + screening: screening occurs and then visit 1 is baseline, visit 2 is at 3 months, visit 3 is at 6 months, visit 4 is at 9 months and visit 5 is at 12 months (final visit)

198 (100%) of randomized subjects completed visit 1 (baseline)
165 subjects completed the 3-month visit.
143 subjects completed the 6-month visit.
133 subjects completed the 9-month visit.
129 subjects completed the 12-month visit.

Withdrawals

70 subjects total have withdrawn from the study following randomization (withdrawal rate of 35%, which is higher than the assumed 10% rate for the power analysis). Summary information is presented in the following table 1 for these subjects.

Table 1. Withdrawals following Randomization

Subject Number	Randomized Dose Level	Baseline Visit Date	Date of Withdrawal	Reason given for dropping	Subject willing to come in for follow up
60003	400	5/9/2008	6/22/2009	lost to follow up	No
60004	2400	5/21/2008	4/6/2009	noncompliant (did not complete 24hour urine retest for hypercalciuria)	No
60021	1600	2/3/2009	6/11/2009	lost to follow up	No
60028	400	2/16/2009	9/25/2009	AE/SAE (AE of itching)	No
60030	400	2/17/2009	9/2/2009	scheduling problems	No
60038	400	3/5/2009	11/4/2009	no reason provided	No
60039	800	2/5/2009	6/15/2009	health concern	No
60044	1600	3/19/2009	10/12/2009	scheduling problems	No
60048	400	4/17/2009	12/7/2009	health concern (endocrinologist started her on Vit D3)	No
60049	Placebo	4/22/2009	2/1/2010	no reason provided	
60051	2400	4/24/2009	9/17/2009	scheduling problems	No
60053	1600	5/8/2009	1/1/2010	lost to follow up	No
60060	Placebo	6/11/2009	10/26/2009	lost to follow up	No
60061	800	6/9/2009	10/19/2009	scheduling problems	No
60205	1600	4/29/2008	10/10/2008	lost to follow up	No
60211	1600	5/30/2008	4/7/2009	lost to follow up	No
60213	800	6/6/2008	9/12/2008	health concern	Yes
60215	Placebo	6/9/2008	10/17/2008	lost to follow up	No
60222	800	6/30/2008	3/23/2010	lost to follow up	
60224	400	7/7/2008	8/14/2009	personal reasons	No
60225	2400	7/7/2008	2/10/2009	lost to follow up	No
60226	2400	8/4/2008	11/12/2008	lost to follow up	No
60230	800	2/4/2009	11/9/2009	personal reasons	No
60236	1600	1/28/2009	5/20/2009	personal reasons	No
60241	Placebo	2/3/2009	12/21/2009	personal reasons	No
60245	800	2/6/2009	6/1/2009	lost to follow up	No
60257	800	3/6/2009	4/6/2010	personal reasons	
60258	2400	3/6/2009	4/17/2009	lost to follow up	No
60262	800	5/6/2009	9/17/2009	lost to follow up	No
60270	400	5/18/2009	4/20/2010	health concern	
60403	2400	5/6/2008	10/10/2008	lost to follow up	No
60405	400	5/30/2008	10/10/2008	lost to follow up	No
60406	1600	6/9/2008	3/11/2009	lost to follow up	No

60407	800	6/12/2008	9/25/2008	lost to follow up	No
60408	2400	6/17/2008	1/9/2009	lost to follow up	No
60409	800	6/20/2008	8/27/2008	AE/SAE (GI pain, cardiac issues, constipation)	No
60410	800	7/30/2008	2/27/2009	lost to follow up	No
60411	Placebo	8/26/2008	2/13/2009	lost to follow up	No
60413	2400	9/8/2008	9/23/2008	lost to follow up	No
60414	Placebo	1/27/2009	5/5/2010	lost to follow up	No
60415	800	2/16/2009	12/11/2009	lost to follow up	No
60417	400	3/4/2009	9/11/2009	scheduling problems	No
60419	1600	3/15/2009	5/3/2010	lost to follow up	No
60421	1600	3/24/2009	10/26/2009	lost to follow up	No
60423	2400	3/26/2009	10/23/2009	lost to follow up	No
60424	Placebo	4/3/2009	8/13/2009	lost to follow up	No
60427	800	4/16/2009	11/2/2009	lost to follow up	No
60430	2400	4/28/2009	10/26/2009	lost to follow up	No
60431	400	4/29/2009	5/21/2009	health concern	No
60436	1600	5/20/2009	12/19/2009	lost to follow up	No
60439	800	2/2/2010	5/5/2009	lost to follow up	No
60441	Placebo	3/23/2010	7/13/2010	lost to follow up	No
60443	400	4/5/2010	8/2/2010	lost to follow up	No
60447	400	5/13/2010	9/2/2010	lost to follow up	No
60603	1600	7/14/2008	1/17/2009	lost to follow up	No
60604	800	7/22/2008	5/5/2008	health concern	No
60608	800	9/11/2008	9/10/2009	lost to follow up	No
60609	2400	10/13/2008	1/23/2009	lost to follow up	No
60616	800	5/11/2009	12/15/2009	lost to follow up	No
60618	400	6/19/2009	2/25/2010	Pt doesn’t want to take pills	No
60619	2400	11/5/2009	10/8/2010	lost to follow up	No
60623	400	11/23/2009	3/17/2010	lost to follow up	No
60624	2400	11/25/2009	3/30/2010	lost to follow up	No
60626	0	2/22/2010	4/29/2011	lost to follow up	No
60628	400	3/24/2010	7/12/2010	lost to follow up	No
60632	2400	7/1/2010	3/21/2011	lost to follow up	No
60633	2400	6/28/2010	11/17/2010	lost to follow up	No
60635	800	7/9/2010	1/10/2011	Schedule too busy	No
60640	0	9/24/2010	4/29/2011	lost to follow up	No

Results of Randomization by Strata:
Subjects are randomized to receive dose levels 0-2400. The randomization is stratified by race (African American and Caucasian) and serum 25OHD (Low and High), and was performed in randomly chosen blocks of size 4 or 8. The randomization is given in the following table 2.

Table 2. Randomization by strata (n=198).

	Randomized Drug Assignment				
Stratum	Dose Placebo	Dose 400	Dose 800	Dose 1600	Dose 2400
African Am.	15	16	18	15	15
Low 25OHD	9	8	9	9	6
High 25OHD	6	8	9	6	9
Caucasians	23	21	24	26	25
Low 25OHD	10	10	11	11	12
High 25OHD	13	11	13	15	13
Total	38	37	42	41	40

During randomization of subjects, the following randomization errors had occurred. All were stratification errors. Subjects will be analyzed according to their randomization group; however, their actual serum 25OHD will be used in the dose response modeling.

- Subject 60237 had a serum 25OHD of 11, but was assigned to strata High 25OHD.
- Subject 60607 had a serum 25OHD of 8 and was African American, but was assigned to strata Caucasian, High 25OHD
- Subject 60013 had a serum 25OHD of 14, but was assigned to strata High 25OHD.
- Subject 60406 had a serum 25OHD of 10, but was assigned to strata High 25OHD.

Baseline characteristics of subjects randomized
All subjects combines and also by ethnicity

Baseline Characteristics –all subjects. Baseline characteristics are compared by treatment assignment in table 3. Season
was defined using months of the year. Months 12, 1, and 2 are winter, 3-5 are spring 6-8 are summer and 9-11 are fall.

Table 3. Baseline Characteristics by treatment assignment (n=198) Mean (SD)

	All women (n=198)	Placebo (n=38)	Dose 400 IU/day (n=37)	Dose 800 U n=42)	Dose 1600 IU (n=41)	Dose 2400 IU (n=40)
Age: mean	36.7 (5.9)	36.4 (6.1)	35.6 (6.6)	37.7 (5.5)	36.4 (5.9)	37.2 (5.4)
Weight (lbs):	165.0 (6.1)	165.6 (6.1)	165.9 (6.9)	163.2 (6.2)	164.5 (4.8)	165.9 (6.2)
Height (cm):	82.1 (18.7)	84.5 (19.9)	80.2 (20.8)	83.1 (15.7)	78.7 (17.7)	83.8 (19.8)
BMI:	30.2 (6.6)	30.9 (6.7)	31.0 (8.1)	31.1 (5.8)	28.4 (5.4)	30.0 (6.6)
Race: N (%)						
African American	79 (40%)	15 (39%)	16 (43%)	18 (43%)	15 (37%)	15 (38%)
Caucasian	119 (60%)	23 (61%)	21 (47%)	24 (57%)	26 (63%)	25 (62%)
Smoking status: N (%)						
Current smoker	36 (18%)	5 (13%)	8 (22%)	7 (17%)	9 (22%)	7 (18%)
Former smoker	35 (18%)	5 (13%)	8 (22%)	7 (17%)	7 (17%)	8 (20%)
Non-smoker	127 (64%)	28 (74%)	21 (57%)	28 (66%)	25 (61%)	25 (62%)
Alcohol use: N (%)						
No	54 (27%)	11 (29%)	13 (35%)	9 (21%)	8 (20%)	13 (32%)
Yes	144 (73%)	27 (71%)	24 (65%)	33 (79%)	33 (80%)	27 (68%)
Serum Calcium mg/dl	9.2 (0.3)	9.1 (0.3)	9.2 (0.3)	9.2 (0.3)	9.2 (0.3)	9.1 (0.3)
24h Urine Calcium mg	138.1 (77.4)	131.9 (86.6)	144.9 (73.2)	120.9 (74.6)	158.2 (80.2)	135.2 (70.)
Serum Creatinine: mg/dl	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.7 (0.1)	0.7 (0.1)	0.8 (0.1)
	59.2 (17.5)	61.1 (17.7)	60.4 (13.1)	59.2 (18.1)	60.4 (20.7)	55.1 (17.1)
Serum alkaline phosph	94.8 (15.8)	93.7 (8.4)	92.1 (9.6)	99.3 (25.7)	95.5 (16.7)	92.9 (9.8)
Serum Glucose: mg/dl	19.9 (5.5)	20.6 (6.2)	19.9 (5.2)	18.8 (3.8)	20.0 (6.2)	20.3 (6.0)
Serum AST: IU/ml	17.6 (9.4)	18.6 (9.1)	17.4 (7.5)	16.2 (6.0)	18.1 (11.8)	17.8 (11.3)
Serum ALT: IU/ml	13.4 (4.5)	12.7 (4.1)	13.1 (4.2)	13.8 (4.3)	13.3 (5.1)	14.1 (4.8)
Serum 25OHD ng/ml	36.1 (13.7)	36.7 (12.1)	33.0 (11.8)	38.3 (16.6)	35.9 (14.6)	

Serum PTH: pg/ml						36.4 (12.4)
Dietary Calcium intake (mg)	655 (262)	677 (275)	627 (248)	599 (249)	681 (282)	692 (254)
Dietary Vitamin D intake/day (IU/d)	100 (72)	108 (82)	102 (69)	89 (75)	113 (84)	91 (43)
Current Drug Use: Thiazide use	12 (6%)	2 (5%)	1 (3%)	2 (5%)	3 (7%)	4 (10%)
Diuretic use	1 (0.5%)	1 (3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Season at baseline	49 (25%)	11 (29%)	10 (27%)	9 (21%)	9 (22%)	10 (25%)
Winter	96 (48%)	17 (45%)	18 (49%)	19 (45%)	24 (59%)	18 (45%)
Spring	41 (21%)	4 (11%)	8 (22%)	12 (29%)	7 (17%)	10 (25%)
Summer	12 (6%)	6 (16%)	1 (3%)	2 (5%)	1 (2%)	2 (5%)
Fall						

Table 4. Baseline Characteristics compared by race (n=198) mean (SD)

	All Subjects (n=198)	African American (n=79)	Caucasian (n=119)
Age:yrs	36.7 (5.9)	35.1 (6.0)	37.7 (5.6)
Weight (lbs):)	165.0 (6.1)	164.1 (5.6)	165.6 (6.3)
Height (cm):	82.1 (18.7)	87.0 (20.3)	78.8 (16.9)
BMI:	30.2 (6.6)	32.5 (6.7)	28.8 (6.1)
Smoking status: N (%)			
Current smoker	36 (18%)	11 (14%)	25 (21%)
Former smoker	35 (18%)	12 (15%)	23 (19%)
Non-smoker	127 (64%)	56 (71%)	71 (60%)
Alcohol use: N (%)			
No	54 (27%)	26 (33%)	28 (24%)
Yes	144 (73%)	53 (67%)	91 (76%)
Serum Ca mg/dl:	9.2 (0.3)	9.1 (0.3)	9.2 (0.3)
24 hr Urine Ca (mg):	138.1 (77.4)	114.8 (73.2)	153.6 (76.5)
Serum Creatinine mg/dl	0.8 (0.1)	0.8 (0.1)	0.7 (0.1)
Serum alkaline phosphatase: IU/ml	59.2 (17.5)	61.3 (19.4)	57.9 (16.2)
Serum Glucose: mg/dl	94.8 (15.8)	93.3 (10.8)	95.8 (18.4)
Serum AST: IU/ml	19.9 (5.5)	20.4 (6.0)	19.6 (5.2)
Serum ALT: IU/ml	17.6 (9.4)	17.9 (11.5)	17.4 (7.7)
Serum 25OHD ng/ml	13.4 (4.5)	11.6 (4.1)	14.6 (4.4)
Serum PTH: pg/ml	36.1 (13.7)	40.9 (16.3)	32.9 (10.5)
Dietary Calcium intake (mg)	655 (262)	503 (159)	756 (268)
Current Drug Use:	12 (6%)	8 (10%)	4 (3%)

Thiazide use Diuretic use	1 (0.5%)	0 (0%)	1 (0.8%)

Primary Outcomes

Serum 25OHD
A mixed effects model was used to estimate the dose response curve of serum 25OHD for each race and time point together in one model with subject as a random effect. In fitting the model 198 subjects had serum 25OHD levels at baseline, 142 at 6 months and 129 at 12 months. Model fit was examined with various residual plots and AIC values and the models were determined to fit as well as possible. Significant interaction terms were present between race, dose and time ($p=0.016$), between dose and race ($p=0.010$), and time and race ($p=0.0051$). Since there are significant interactions with race in the model, the African American women and Caucasian women were modeled separately.

African American women 25OHD dose response
For the African American model, 79 subjects had serum 25OHD levels at baseline, 45 at 6 months and 39 at 12 months. The interaction between dose^2 and time ($p=0.78$) and the dose^2 term ($p=0.58$) were not significant, so these terms were excluded from the model. The final model included significant fixed effects time, dose, and the dose by time interaction. The slopes did not differ significantly between the 6 and 12 month curves ($p=0.33$), nor did the intercepts ($p=0.88$).The estimated model can be found in Table 5 and Figure 2.
Caucasian women 25OHD dose response
For the Caucasian model, 119 subjects had serum 25OHD levels at baseline, 97 at 6 months and 90 at 12 months. The interaction between dose^2 and time ($p=0.18$) and dose^2 terms were also not significant ($p=0.070$), so these terms were excluded from the model. The final model included significant fixed effects time, dose, and the dose by time interaction (all $p<0.0001$). The slopes did not differ significantly between the 6 and 12 month curves ($p=0.35$), but the intercepts marginally did ($p=0.045$). The estimated model can be found in Table 5 and Figure 3.

Table 5. Dose response mixed effects model, estimating the dose response of serum 25OHD separately for each race.

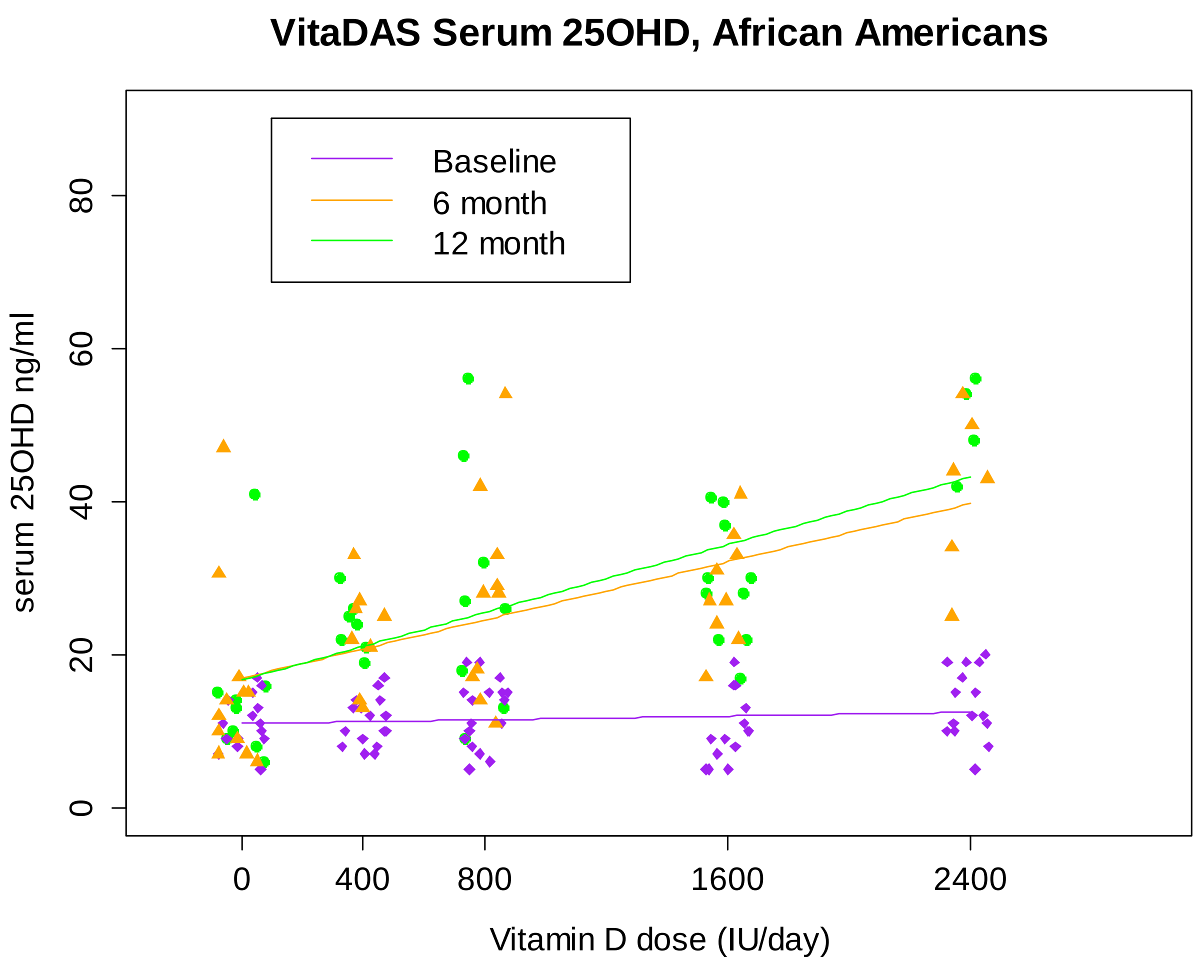
	African American women						Caucasian wome			
				95% confidence interval						
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value	Effect		β estimate	S
Intercept		16.622	1.779	13.080	20.165		Intercept		24.345	1
Time	Baseline	-5.652	1.848	-9.330	-1.974	0.0009	Time	Baseline	-10.074	1
	6 months	0.295	1.936	-3.558	4.148			6 months	2.737	1
	12 months	0	-	-	-			12 months	0	

Dose	1000 IU increase	11.061	1.480	8.116	14.007	<0.0001	Dose	1000 IU increase	6.136	0
Dose*Time	Baseline	-10.451	1.527	- 13.491	-7.411	<0.0001	Dose*Time	Baseline	-5.844	0
	6 months	-1.583	1.628	-4.823	1.657			6 months	-0.900	0
	12 months	0	-	-	-			12 months	0	

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

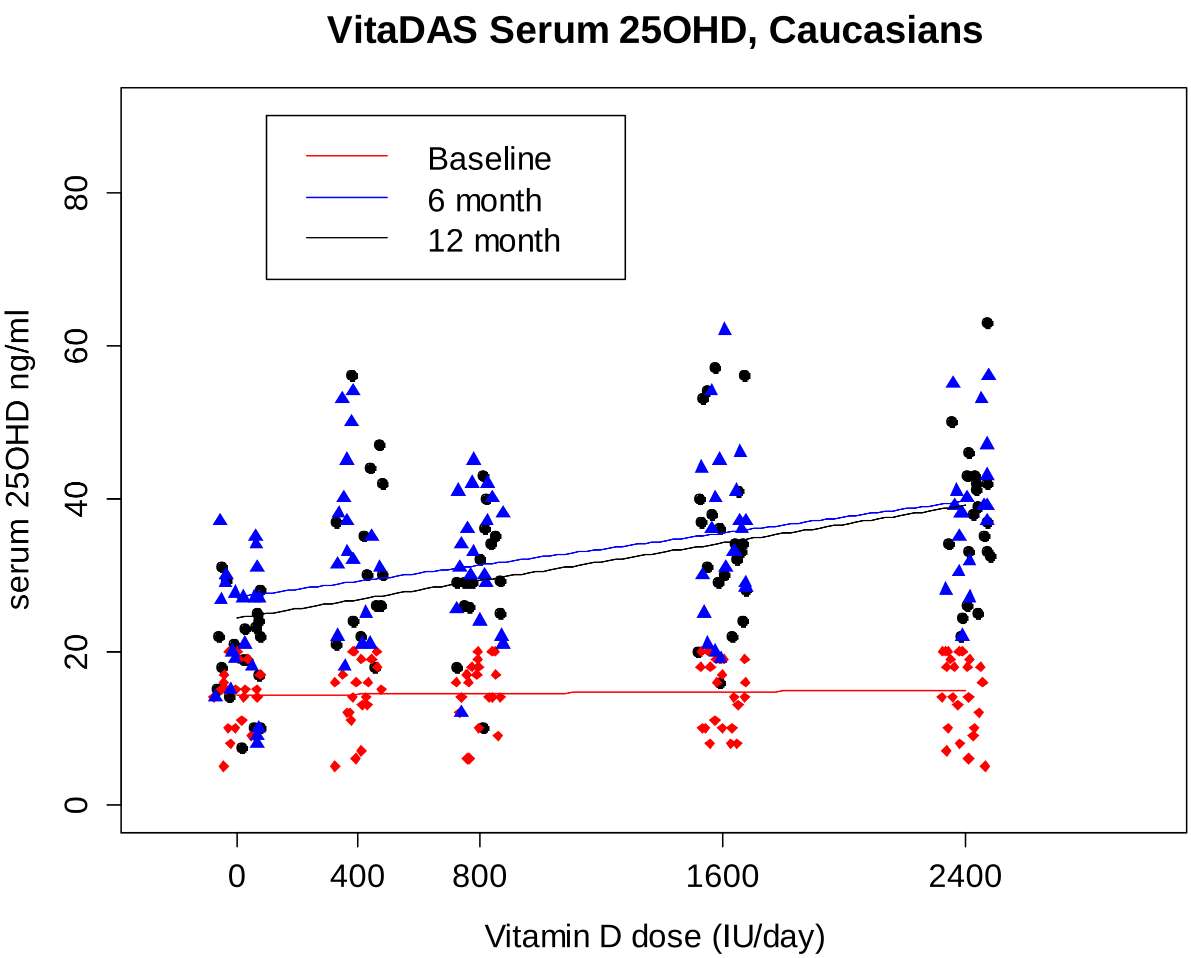
e fitted dose response curves for serum 25OHD in African American women are shown in figure 2. As seen in figure 2 and in table 5 the coefficients for time and dose*time at 6 months are not significantly different from that at 12 months based on the confidence intervals (table 5) and pairwise p-values between 6 and 12 months (p=0.88 and p=0.33 respectively).

Figure 2. Fitted dose response curves for serum 25OHD at each time point – African Americans



The fitted dose response curves for serum 25OHD in Caucasian women are shown in figure 3. As seen in figure 3 and in table 5 the slopes did not differ significantly between the 6 and 12 month curves (p=0.35), but the intercepts marginally did (p=0.045).

Figure 3. Fitted dose response curves for serum 25OHD at each time point - Caucasians



The following 3 plots show the dose response curves for serum 25OHD at baseline, 6 and 12 months, with African American and Caucasian women on the same plot.

Figure 4. Baseline serum 25OHD

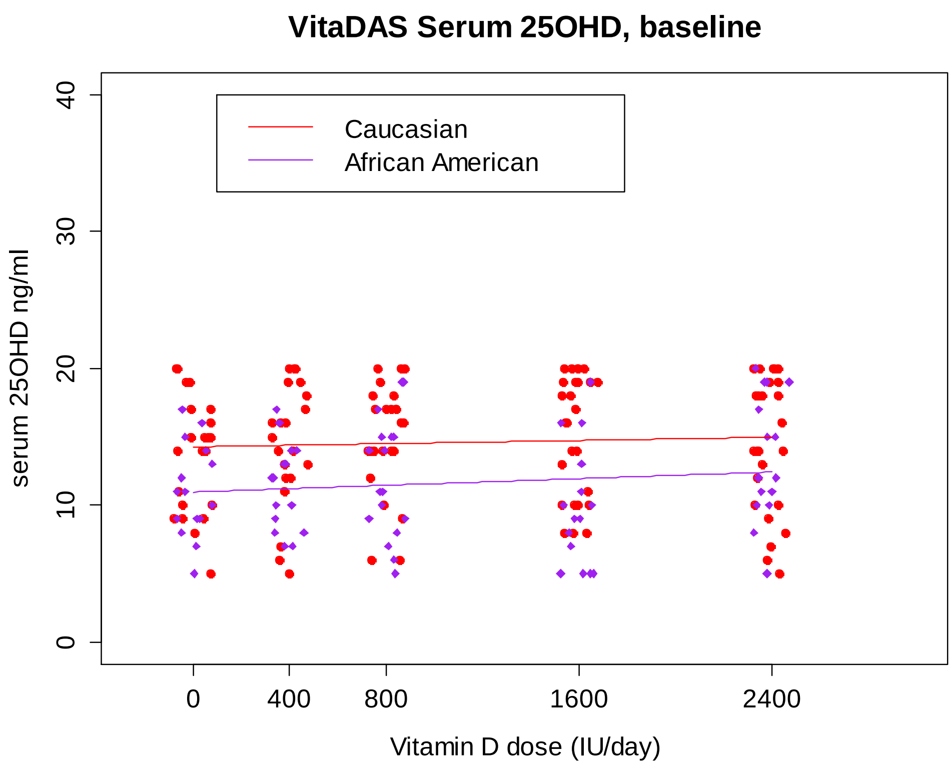


Figure 5. The 6 and 12 month serum 25OHD

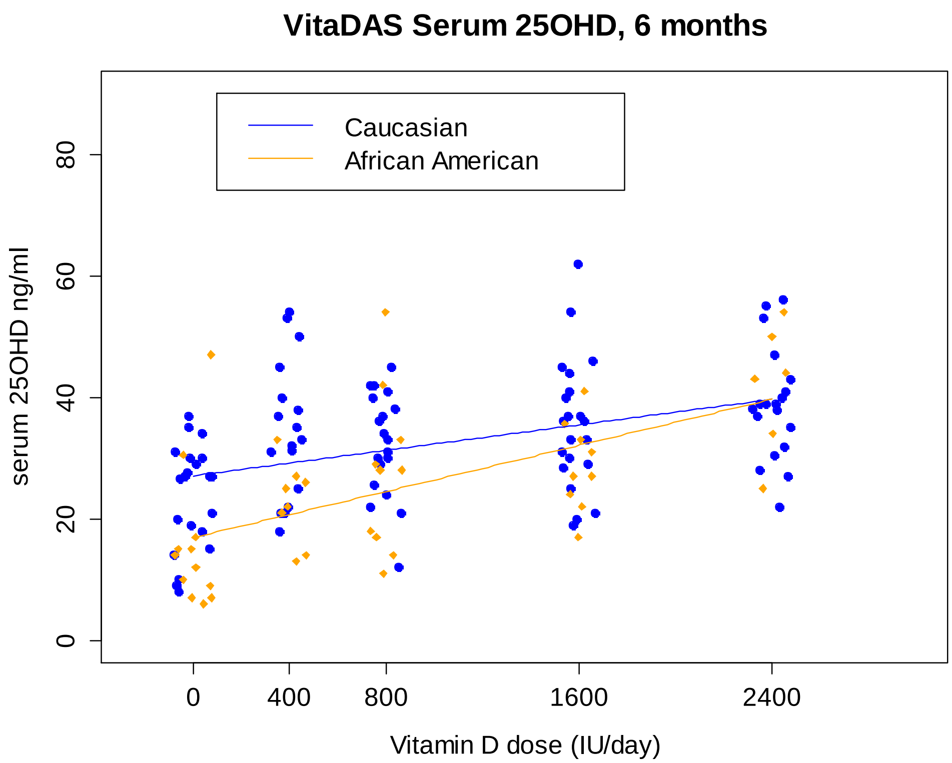
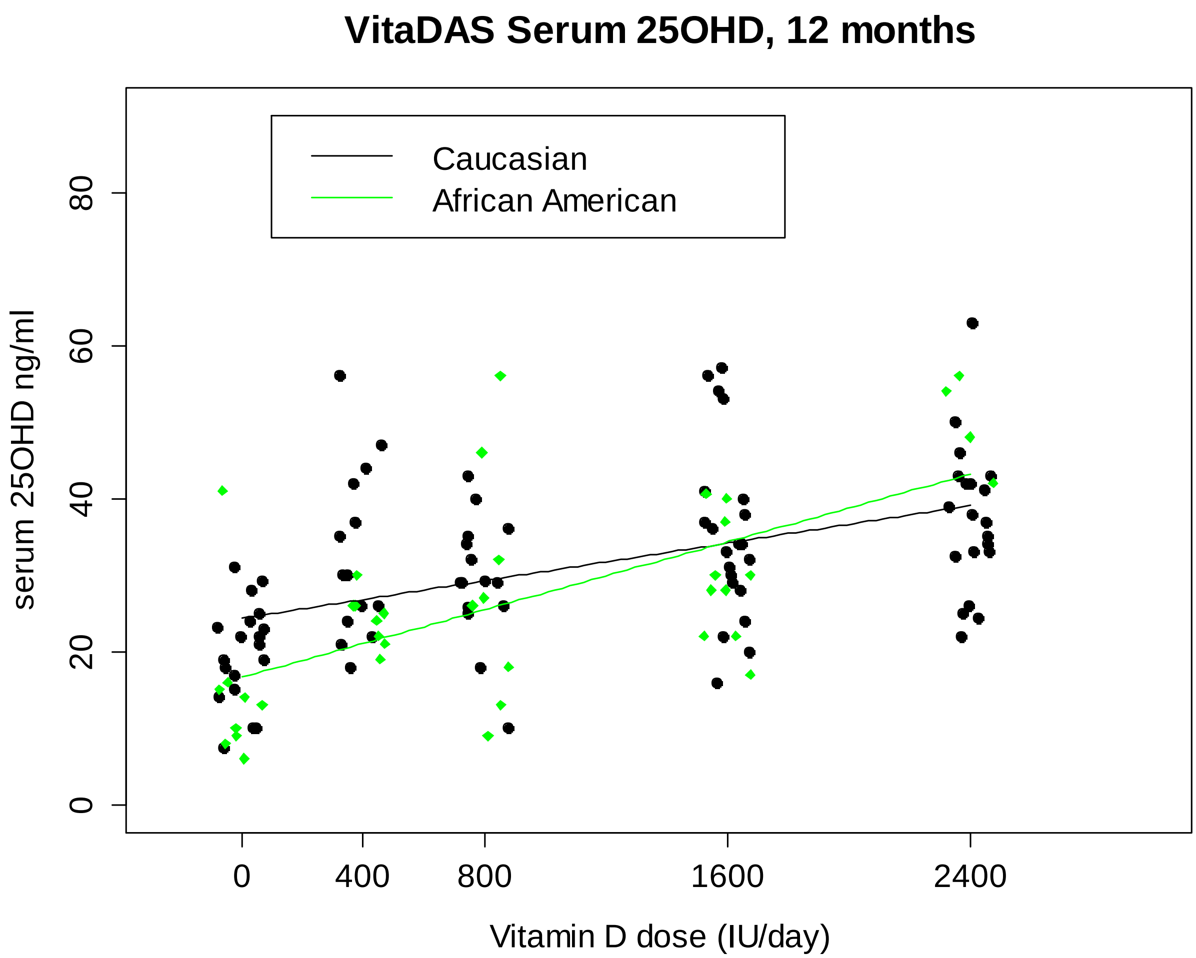


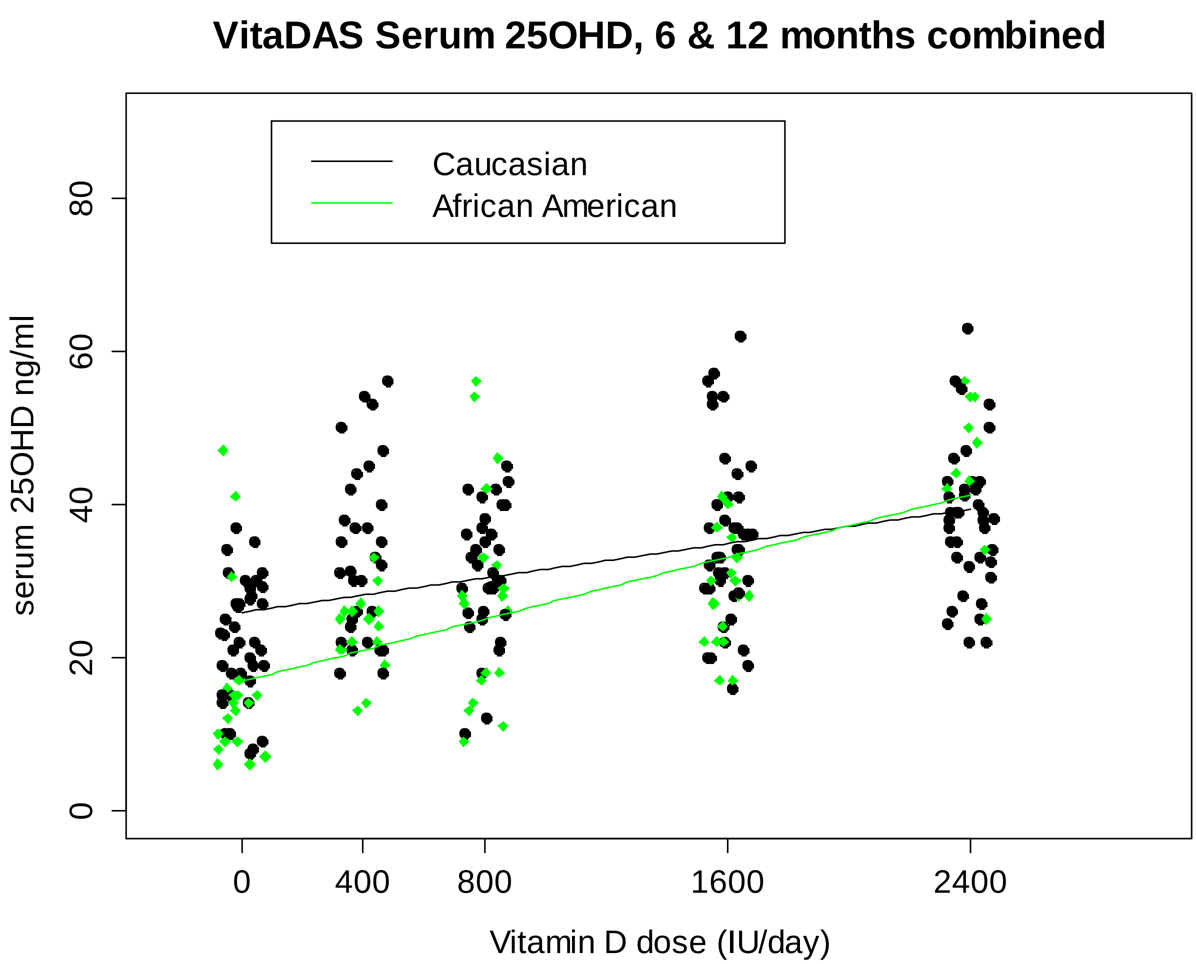
Figure 6. The 12 month serum 25OHD



Since the 6 and 12 month time points were not significantly different we fit combined models for Caucasians and African Americans. The fitted lines for the Caucasian and African American subjects for 6 and 12 month time point combined are following.

Caucasian: Serum 25OHD = 25.8422 + 5.6261*Dose/1000
African Americans: Serum 25OHD = 16.8285 + 10.1577*Dose/1000

Figure 7. The 6 and 12 month data combined.



Prediction intervals

Cutpoint of 30 ng/ml

The mixed effects model was used to determine the 95% prediction limits for the 6 and 12 month serum 25OHD levels (see table 6). The lower prediction limits reach 30 ng/ml at a dose of 2400 IU, indicating that 97.5% of individuals will obtain a level of serum 25OHD of 30 ng/ml at a dose of 2400 IU; therefore, the estimated RDA is near 2400 IU vitamin D_3. African American women reach above 30 ng/ml at a dose between 1600 and 2400-this is their estimated RDA. To estimate the dose that obtains the EAR, we want 50% of new individuals to obtain a serum 25OHD level greater than 30 ng/ml. At a dose of 800 IU the median predicted value will be above 30 ng/ml for a new individual in the Caucasian population. A dose of 800 IU should obtain a serum 25OHD of greater than 30 ng/ml in 50% of Caucasian women. At a dose of 1600 IU the median predicted value will be above 30 ng/ml for a new individual in the African American population. A dose of 1600 IU should obtain a serum 25OHD of greater than 30 ng/ml in 50% of African American women.

Cutpoint of 20 ng/ml for estimating the RDA and EAR

The lower prediction limits reach 20 ng/ml at a dose of 400 IU in Caucasian women, indicating that 97.5% of individuals will obtain a level of serum 25OHD of 20 ng/ml at a dose of 400 IU this is their estimated RDA. For African American women the lower prediction limit reaches above 20 ng/ml at a dose between 800 and 1600 IU and this is their estimated RDA.

To estimate the dose that obtains the EAR, we want 50% of new individuals to obtain a serum 25OHD level greater than 20 ng/ml. A dose of
0-400 IU should obtain a serum 25OHD of greater than 20 ng/ml in 50% of Caucasian women. At a dose of 400 IU the median predicted value will be above 20 ng/ml for a new individual in the African American population.

Figure 8 shows the 12 month serum 25OHD data with the fitted line from the mixed-effects model with the 95% bootstrapped prediction limits for the African American women and figure 9 shows the same for the Caucasian women.

Table 6. Bootstrapped 95% prediction intervals determined with mixed effects models

	African American						Caucasian					
	6 month			12 month			6 month			12 month		
Dose	Lower limit	Median	Upper limit	Lower limit	Median	Upper limit	Lower limit	Median	Upper limit	Lower limit	Median	Upper limit
Placebo	10.29	15.37	32.5	10.12	15.25	32.05	16.65	26.31	31.16	13.98	23.53	28.27
400	16.9	21.08	26.49	17.31	21.42	26.73	22.91	30.24	42.67	20.55	27.81	40.33
800	16.18	24.39	39.6	17.02	25.33	40.69	21.63	31.85	38.42	19.54	29.8	36.39
1600	22.34	30.36	36.32	24.24	32.5	38.43	25.93	35.04	47.41	24.61	33.72	46.06
2400	32.65	40.32	49.2	36.15	43.59	52.13	30.94	39.21	49.98	30.19	38.54	49.69

Figure 8. The 12 month serum 25OHD with 95% bootstrapped prediction limits – African Americans

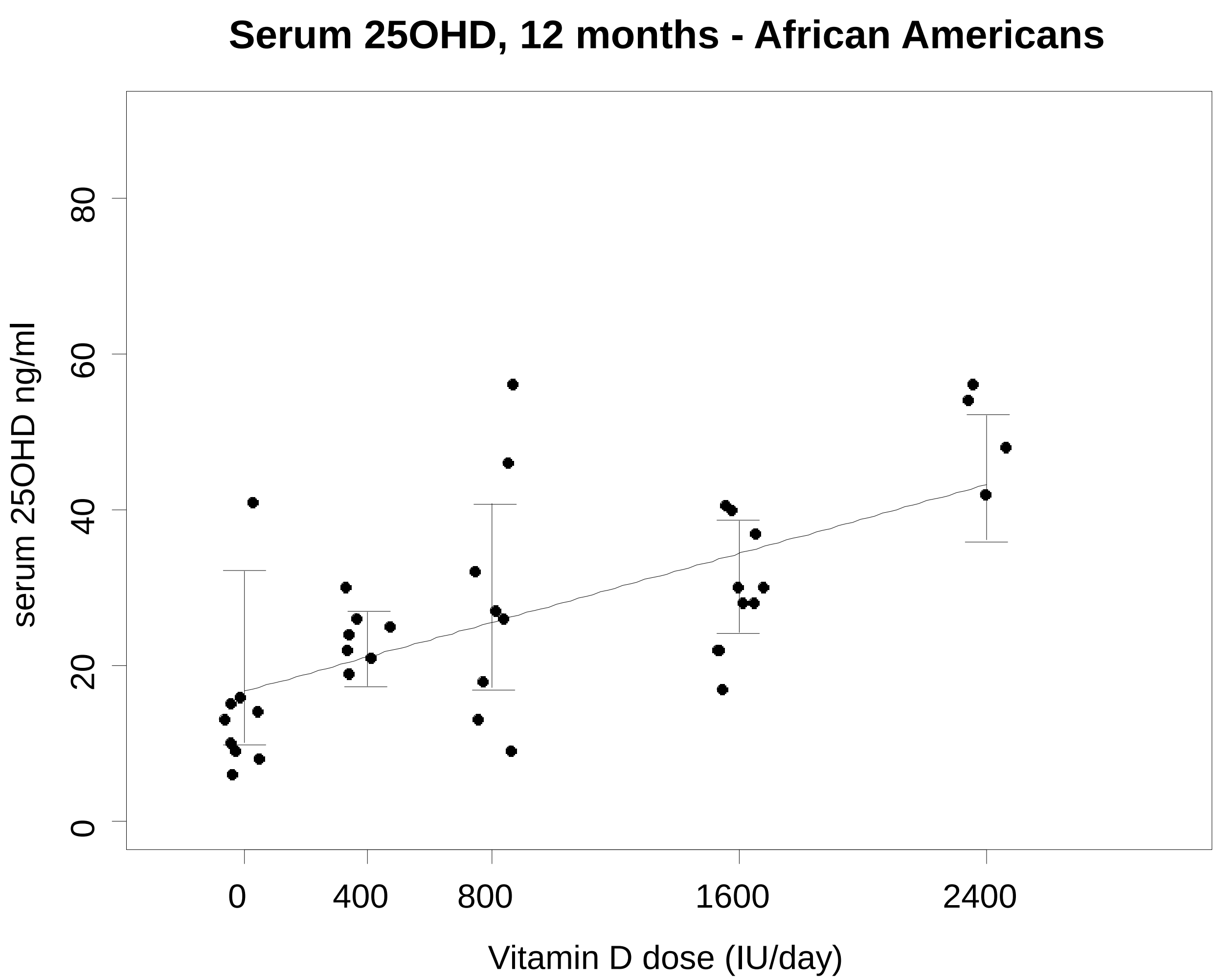
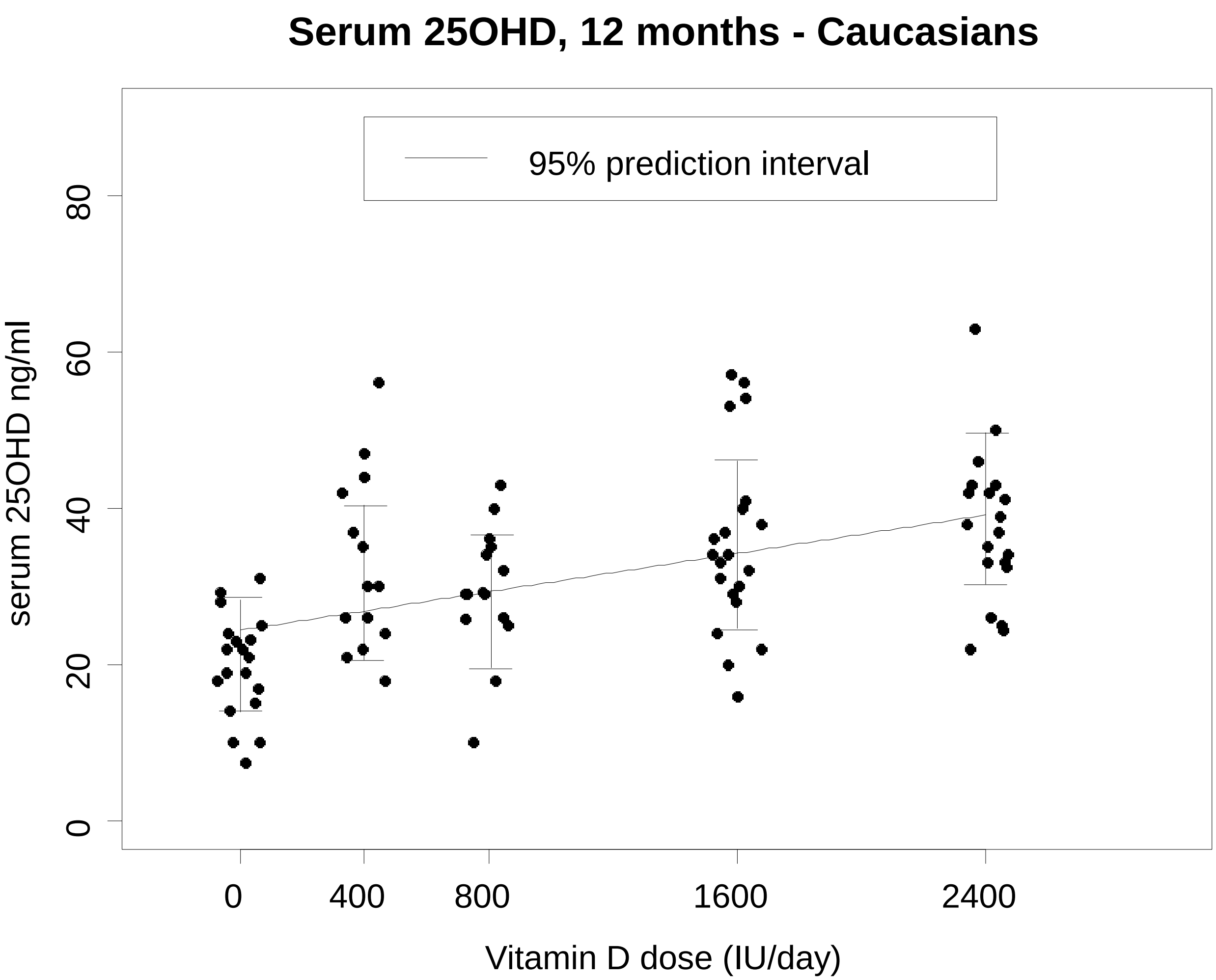


Figure 9. The 12 month serum 25OHD with 95% bootstrapped prediction limits – Caucasian women.



Serum PTH

A mixed effects model was used to estimate the dose response curve of serum PTH for each race and time point together in one model with subject as a random effect. In fitting the model 198 subjects had serum 25OHD levels at baseline, 139 at 6 months and 128 at 12 months. Model fit was examined with various residual plots and AIC values and the models were determined to fit well. PTH on the logarithmic scale fit the best. There appeared to be some marginal interactions with race, therefore, the African American women and Caucasian women were modeled separately.

African American women PTH dose response

For the African American model, 79 subjects had serum 25OHD levels at baseline, 44 at 6 months and 39 at 12 months. The interaction between dose^3 and time (p=0.24) and dose^2 and time (p=0.41) were not significant, so these terms were excluded from the model. Also dose^3 (p=0.076) and dose^2 terms (p=0.17) were also not significant. The interaction between dose and time was not significant (p=0.11). The final model included effects for time (p=0.0002) and dose (p=0.83). log PTH decreased significantly from baseline to 6 months (p=0.0056) by 0.079, which corresponds to a 6.31 decrease on the original scale (by exponentiating the mean levels at baseline and 6 months and taking the difference), but did not differ significantly between 6 and 12 months (p=0.57). The estimated model can be found in Table 7 and Figure 10.

Caucasian women PTH dose response

For the Caucasian model, 119 subjects had serum 25OHD levels at baseline, 95 at 6 months and 89 at 12 months. The interaction between dose^3 and time (p=0.83) and dose^2 and time (p=0.85) were not significant, so these terms were excluded from the model. Also dose^3 (p=0.50) and dose^2 terms (p=0.20) were also not significant. The interaction between dose and time was not significant (p=0.11). The final model included effects for time (p<0.0001) and dose (p=0.63). log PTH decreased significantly from baseline to 6 months (p<0.0001) by 0.085, which corresponds to a 5.56 decrease on the original scale (by exponentiating the mean levels at baseline and 6 months and taking the difference), but did not differ significantly between 6 and 12 months (p=0.16). The estimated model can be found in Table 7 and Figure 11.

Table 7. Dose response mixed effects model, estimating the dose response of log(serum PTH) separately for each race.

	African American women						Caucasian women			
				95% confidence interval						
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value	Effect		β estimate	SE
Intercept		1.47540	0.03474	1.40620	1.54450		Intercept		1.44360	0.0207
Time	Baseline	0.10710	0.02599	0.05537	0.15880	0.0002	Time	Baseline	0.05956	0.0135
	6 months	0.02784	0.02732	-0.02652	0.08220			6 months	-0.02520	0.0138
	12 months	0	-	-	-			12 months	0	-
Dose	1000 IU increase	-0.00484	0.02246	-0.04954	0.03985	0.83	Dose	1000 IU increase	-0.00662	0.0135

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

Figure 10. Fitted dose response curves for PTH at each time point – African Americans

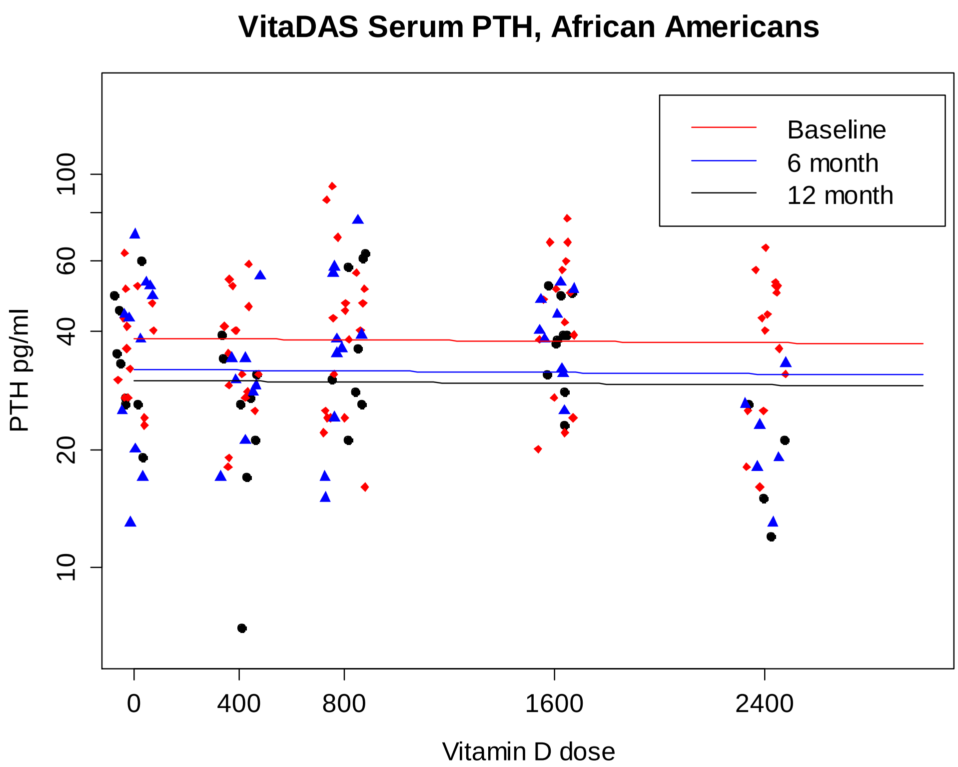
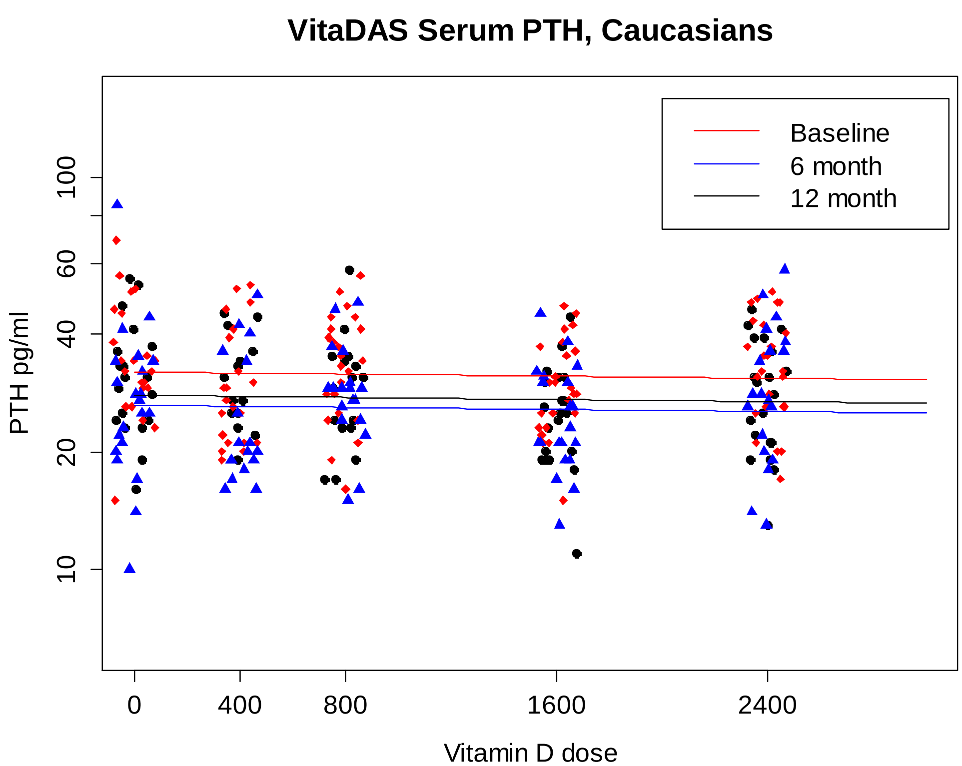


Figure 11. Fitted dose response curves for PTH at each time point - Caucasians



Missing data
To formally test whether the missing data mechanism can be assumed to be MCAR, we used a mixed effects regression model with indicator variables to denote the missing data in the model. 4 missing data patterns were observed and can be identified as detailed in Tables 8 and 9. "Observed" indicates that the outcome variable (serum 25OHD or PTH) was observed for that visit and "Missing" indicates that the outcome variable was not observed for that visit. Since there were only 2

Table 8. Missing Data Patterns for Testing for MCAR – Serum 25OHD

Baseline	Month 6	Month 12	Missing Data Pattern	N
Observed	Observed	Observed	P0	127
Observed	Observed	Missing	P1	15
Observed	Missing	Observed	P2	2
Observed	Missing	Missing	P3	54

Table 9

Missing Data Patterns for Testing for MCAR – Serum PTH

Baseline	Month 6	Month 12	Missing Data Pattern	N
Observed	Observed	Observed	P0	126
Observed	Observed	Missing	P1	13
Observed	Missing	Observed	P2	2
Observed	Missing	Missing	P3	57

For serum 25OHD the missing data mechanism test showed that we could assume a MCAR model for the African American women, since none of the indicator variables for pattern were significant in the model (all $p>0.2$), but for the Caucasian women the missing data pattern was non-ignorable (see table 10). For serum PTH none of the indicator variables for pattern were significant in the model for both African American and Caucasian women (all $p>0.3$).

Table 10. Test for MCAR in a dose response mixed effects model, estimating the dose response of serum 25OHD separately for each race.

	African American women						Caucasian women						
				95% confidence interval							95% confidence interval		
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value	Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value
Intercept		15.378	2.348	10.700	20.056		Intercept		21.764	2.229	17.349	26.179	
Missing Pattern	P0	1.424	1.817	-2.191	5.039	0.44	Missing Pattern	P0	2.241	1.940	-1.587	6.068	0.25
	P1	1.651	2.851	-4.022	7.325	0.56		P1	7.506	3.079	1.430	13.581	0.016
	P2	0.812	6.627	-12.376	13.999	0.90		P2	-1.789	7.168	-15.931	12.353	0.80
	P3	0	-	-	-			P3	0	-	-	-	
Time	Baseline	-5.400	1.881	-9.142	-1.657	0.0020	Time	Baseline	-10.126	1.345	-12.780	-7.471	<0.0001
	6 months	0.283	1.940	-3.578	4.143			6 months	2.476	1.364	-0.215	5.166	
	12 months	0	-	-	-			12 months	0	-	-	-	
Dose	1000 IU increase	11.136	1.486	8.179	14.092	<0.0001	Dose	1000 IU increase	6.439	0.942	4.581	8.296	<0.0001
Dose*Time	Baseline	-10.378	1.529	-13.421	-7.335	<0.0001	Dose*Time	Baseline	-5.716	0.947	-7.585	-3.848	<0.0001
	6 months	-1.593	1.628	-4.833	1.647			6 months	-0.806	0.976	-2.732	1.120	
	12 months	0	-	-	-			12 months	0	-	-	-	

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

Multivariate model table 11
Total calcium is calculated as average dietary calcium from baseline and 12 months, plus calcium supplementation*average compliance. BMI was defined using the WHO categories (BMI < 25, 25-29.9, >=30). BMI distribution by dose is shown below. Since the African American women have so few subjects in the lower two BMI categories they have been combined in the multivariate analysis.

African Americans

	Placebo	Dose 400	Dose 800	Dose 1600	Dose 2400	Total
BMI <25	2 (13%)	2 (12%)	2 (11%)	2 (13%)	1 (7%)	9 (11%)
BMI 25-29.5	3 (20%)	3 (19%)	7 (39%)	6 (40%)	4 (27%)	23 (29%)
BMI >=30	10 (67%)	11 (69%)	9 (50%)	7 (47%)	10 (67%)	47 (59%)

Caucasians

	Placebo	Dose 400	Dose 800	Dose 1600	Dose 2400	Total
BMI <25	7 (30%)	9 (43%)	4 (17%)	11 (42%)	6 (24%)	37 (31%)
BMI 25-29.5	6 (26%)	5 (24%)	7 (29%)	7 (27%)	10 (40%)	35 (29%)
BMI >=30	10 (43%)	7 (33%)	13 (54%)	8 (31%)	9 (36%)	47 (40%)

The mixed effects models of dose response described above were adjusted for clinically important covariates. Covariates included in the model selection were: age, BMI category (<30, >=30), total calcium intake, smoking status, alcohol use, and serum creatinine. For the Caucasian model, indicator variables for the dropout time were included in order to take into account the missing data mechanism. Age and total calcium intake were highly correlated (r=0.82) in Caucasian women and moderately correlated in African American women (r=0.47).
African American multivariate models
Serum 25OHD

Interactions between dose and covariates were explored, and there were no significant interactions, including between dose and BMI category (p=0.23). The estimated model is shown in table 11. The model is similar to the original dose response model after adjusting for clinical covariates, and none of the clinical covariates were significant predictors of serum 25OHD in African American women.

Serum PTH

Interactions between dose and covariates were explored, and there were no significant interactions. The estimated model is shown in table 11. The model is similar to the original dose response model after adjusting for clinical covariates. BMI category was a marginal predictor of $\log_{10}$(PTH) in the multivariate (MV) model (p=0.042) and season was a significant predictor (p=0.0099).

Table 12 Multivariate mixed effects models of serum 25OHD and PTH – African American women

		Serum 25OHD					Log_{10} (PTH)				
				95% confidence interval					95% confidence interval		
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value	β estimate	SE	Lower limit	Upper limit	Overall P-value
Intercept		12.064	7.3176	-2.538	26.6659		1.2008	0.1706	0.8604	1.5412	
Season	Winter	1.7883	3.1132	-4.4084	7.9849	0.69	0.07731	0.0732	-0.06836	0.223	0.0099
	Spring	0.9464	2.1176	-3.2686	5.1614		0.1647	0.05028	0.06463	0.2648	
	Summer	2.7407	2.4473	-2.1306	7.6121		0.08406	0.05802	-0.03141	0.1995	
	Fall	0	-	-	-		0	.	.	.	
age	1 year increase	-0.04129	0.1242	-0.2885	0.2059	0.74	0.002674	0.002954	-0.0032	0.008552	0.37
BMI	< 30	2.2489	1.5276	-0.7918	5.2896	0.15	-0.07473	0.03622	-0.1468	-0.00265	0.042
	>=30	0	-	-	-		0	.	.	.	
Smoking status	Currently	-1.2265	2.2476	-5.7001	3.2472	0.52	-0.06131	0.05271	-0.1662	0.04358	0.35
	Formerly	1.9512	2.1701	-2.3682	6.2706		0.0328	0.05118	-0.06905	0.1347	
	Never	0	-	-	-		0	.	.	.	
Alcohol use	No vs. Yes	1.5035	1.6459	-1.7726	4.7796	0.36	-0.01226	0.03901	-0.08989	0.06536	0.75
Total calcium intake (mg)	1 mg increase	0.002844	0.003367	-0.00386	0.009546	0.40	0	.	.	.	0.97
Serum Creatinine (mg/dl)	1 mg/dl increase	1.737	7.0496	-12.2949	15.769	0.81	3.31E-06	0.000078	-0.00015	0.00016	0.42
Time	Baseline	-5.7027	1.8597	-9.4042	-2.0011	0.0010	0.1328	0.1655	-0.1965	0.4622	<0.0001

	6 months	0.1906	1.9289	-3.6487	4.03		0.1109	0.02595	0.05931	0.1626	
	12 months	0	-	-	-		0.0288	0.02702	-0.02498	0.08257	
Dose	1000 IU increase	10.3911	1.4919	7.4216	13.3606	<0.0001	0	.	.	.	0.71
Dose*Time	Baseline	-10.2074	1.5284	-13.2497	-7.1652	<0.0001					
	6 months	-1.4846	1.6237	-4.7165	1.7474						
	12 months	0	-	-	-						

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

Caucasian multivariate models

Serum 25OHD

Interactions between dose and covariates were explored, and there were significant interactions between dose and age and dose and BMI. Serum creatinine is also a significant predictor in the model. The estimated model is shown in table 12. To interpret the dose*age interaction, if dose is held fixed, for every year increase in age, serum 25OHD is decreased by 0.29 ng/ml (95% CI:-0.55 to -0.036). To interpret the dose*BMI interaction, on average, the rate of change in serum 25OHD with dose is 3.3 ng/ml greater in a subject with BMI <25 than that of a subject with BMI >=30 (95% CI: 0.03 to 6.6). A 0.1 mg/dl increase in serum creatinine results in a 1.6 ng/ml increase in serum 25OHD (95% CI: 0.52 to 2.7).

Table 13 Multivariate mixed effects models of serum 25OHD– Caucasian women

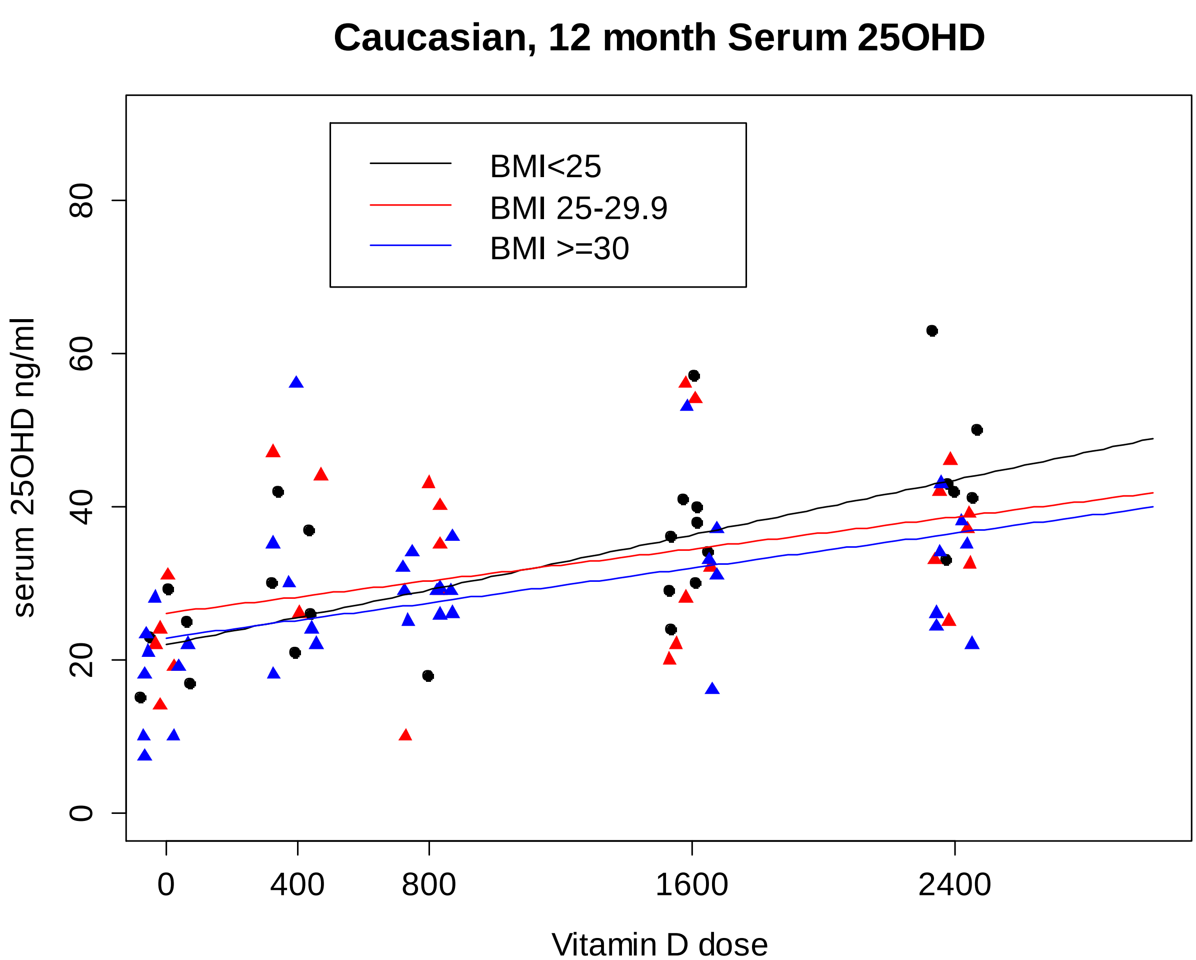
		Serum 25OHD (ng/ml)				
				95% confidence interval		
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value
Intercept		0.4194	7.9627	-15.3745	16.2134	
Dropout time	6 months	-0.7381	2.0179	-4.7194	3.2432	0.71
	12 months	5.785	2.619	0.6177	10.9524	0.028
Season	Winter	0.08483	1.6919	-3.2533	3.4229	0.64
	Spring	1.2031	1.6593	-2.0708	4.4769	
	Summer	0	-	-	-	
Age	1 year increase	0.1345	0.1479	-0.1573	0.4262	0.36
BMI	<25	-0.5865	2.2821	-5.0891	3.916	0.24
	25-29.9	3.2602	2.2284	-1.1366	7.6569	
	>=30	0	-	-	-	
Smoking status	Currently	-0.9393	1.5579	-4.0131	2.1345	0.72
	Formerly	0.5854	1.5289	-2.4312	3.6019	
	Never	0	-	-	-	
Alcohol use	No vs. Yes	-0.8974	1.3503	-3.5615	1.7668	0.51
Total calcium intake (mg)	1 mg increase	0.005698	0.00358	-0.00136	0.01276	0.11
Serum Creatinine (mg/dl)	1 mg/dl increase	16.2356	5.6082	5.1706	27.3007	0.0043
Time	Baseline	-10.0836	1.3377	-12.7229	-7.4443	<0.0001
	6 months	2.521	1.3567	-0.1557	5.1977	
	12 months	0	-	-	-	
Dose		16.5678	5.1666	6.374	26.7617	0.0020
Dose*Time	Baseline	-5.7754	0.9412	-7.6324	-3.9183	<0.0001
	6 months	-0.8231	0.9702	-2.7373	1.0911	

	12 months	0	-	-	-	
Dose*Age		-0.2919	0.1295	-0.5473	-0.03647	0.025
Dose*BMI	<25	3.2922	1.6529	0.03111	6.5533	0.065
	25-29.9	-0.3429	1.6233	-3.5458	2.8599	
	>=30	0	-	-	-	

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

Figure 12 shows the 25OHD dose response curve for Caucasian women at 12 months by BMI. These curves are fit from the multivariate model, assuming all other factors are for an average woman on study, age 37.7, never smoker, alcohol user, total calcium intake of 947 mg, and serum creatinine of 0.7 mg/dl. Different assumptions for the covariates would shift the curves up or down, but would not change the shape of the curves.

Figure 12. Serum 25OHD dose response curve by BMI at 12 months



Serum PTH

Interactions between dose and covariates were explored, and there were significant interactions between dose and smoking status. Season and age are also significant predictors in the model. The estimated model is shown in Table 13. To interpret the dose*smoking interaction, if dose is held fixed, on average a subject who is a current smoker has significantly higher PTH than a never smoker (by 0.09 on the $\log_{10}$ scale). A 1-year increase in age results in a 0.0056 $\log_{10}$ increase in PTH (95% CI: 0.0020 to 0.0094). Subjects randomized in the winter had a significantly lower log PTH than those randomized in the spring (mean $\log_{10}$ difference=-0.0649, 95% CI: -0.1222 to -0.0077, p=0.022).

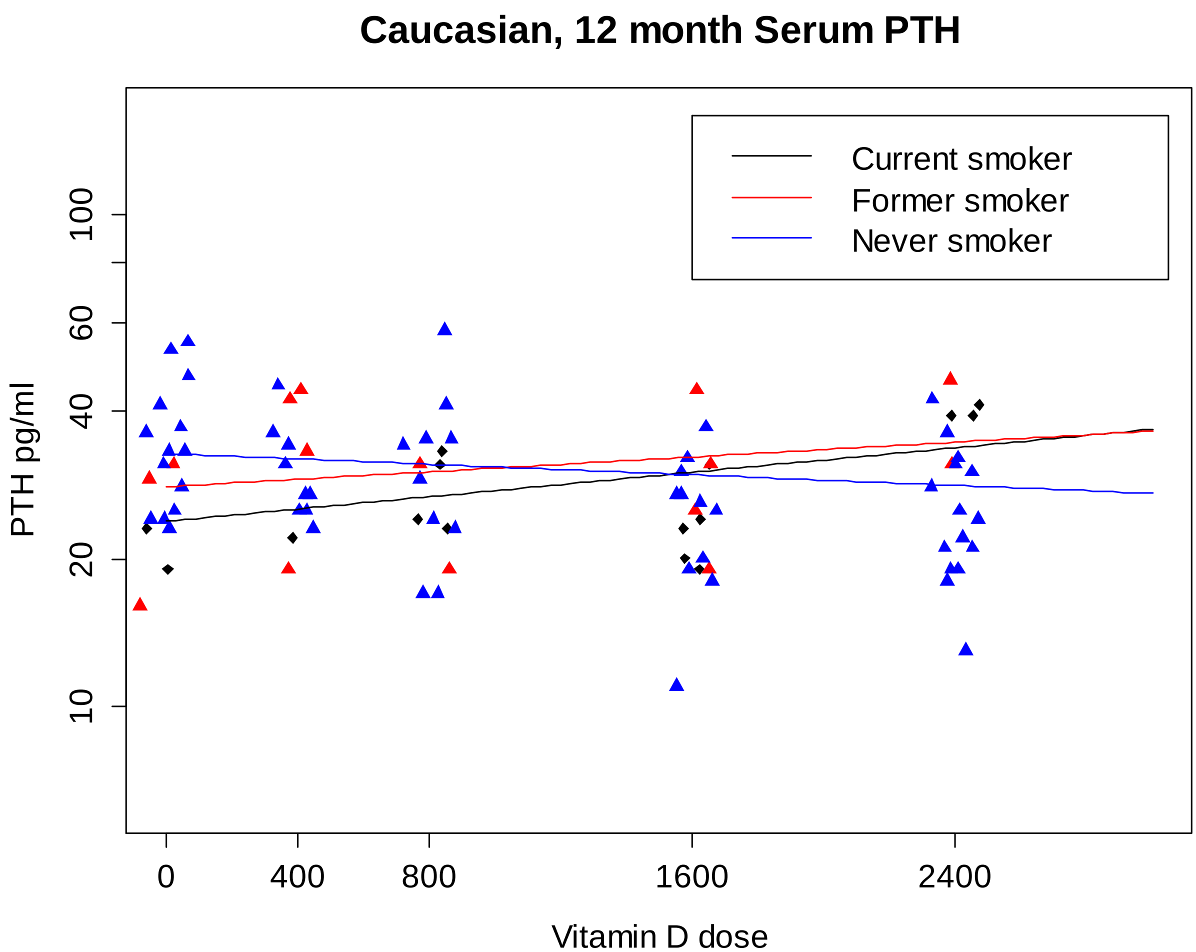
Table 14. Multivariate mixed effects models of serum PTH– Caucasian women

		Log_{10}(PTH)				
				95% confidence interval		
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value
Intercept		1.3514	0.1226	1.1083	1.5944	
Season	Winter	-0.01624	0.03062	-0.07666	0.04418	0.023
	Spring	0.04875	0.02976	-0.00997	0.1075	
	Summer	0	-	-	-	
Age	1 year increase	0.005682	0.001878	0.001977	0.009388	0.0028
BMI	>25	-0.04016	0.02619	-0.09184	0.01151	0.11
	25-29.9	-0.05125	0.02528	-0.1011	-0.00137	
	>=30	0	-	-	-	
Smoking status	Currently	-0.1634	0.04484	-0.2519	-0.07494	0.0011
	Formerly	-0.07328	0.0421	-0.1563	0.009784	
	Never	0	-	-	-	
Alcohol use	No vs. Yes	-0.0214	0.02442	-0.06959	0.02679	0.38
Total calcium intake (mg)	1 mg increase	-0.00002	0.000061	-0.00013	0.000104	0.80
Serum Creatinine (mg/dl)	1 mg/dl increase	-0.06324	0.1017	-0.2639	0.1375	0.53
Time	Baseline	0.06116	0.01347	0.03459	0.08774	<0.0001
	6 months	-0.02438	0.01378	-0.05157	0.002812	
	12 months	0	-	-	-	
Dose	1000 IU increase	-0.03772	0.01445	-0.06623	-0.00922	0.26
Dose* Smoking status	Currently	0.09136	0.03232	0.02759	0.1551	0.0055
	Formerly	0.0717	0.03249	0.007591	0.1358	
	Never	0	-	-	-	

*Dose was divided by 1000, to fit the models. To estimate the outcome variable use doses 0, 0.4, 0.8, 1.6, and 2.4 in the models above to correspond to doses Placebo, 400, 800, 1600, and 2400 IU.

Figure 13 shows the PTH dose response curve for Caucasian women at 12 months by Smoking status. These curves are fit from the multivariate model, assuming all other factors are for an average woman on study, age 37.7, BMI>=30, alcohol user, total calcium intake of 947 mg, and serum creatinine of 0.7 mg/dl. Different assumptions for the covariates (other than creatinine) would shift the curves up or down, but would not change the shape of the curves.

Figure 13. Serum PTH dose response curve by smoking status at 12 months



TASK 3 (i) The effect of Vitamin D on Calcium Absorption in young women.

This task is completed

Summary of results

1,25 dihydroxyvitamin D (1,25$(OH)_2$D) is the major hormone that controls intestinal calcium absorption. Serum 1,25 $(OH)_2$D decreases when serum 25 hydroxyvitamin D (25OHD) falls below <10ng/ml but it is not known at what serum 25OHD level malabsorption of calcium can occur. Based on cross sectional data, one study suggested that a threshold for normal calcium absorption occurred at a serum 25OHD level of 30 ng/ml but in another study it was 5-10ng/ml. We performed a longitudinal study of different doses of vitamin D on absorption to measure the effect on calcium absorption and look for a threshold effect.

198 Caucasian and African American women, ages 25-45 years, were randomized to a 12 month double blind study of vitamin D3 - 400, 800, 1600, 2400, or placebo. Calcium intake was increased to 1200-1400mg/day from an average of 655 mg/day. Main inclusion criteria was that subjects had serum 25OHD level < 20ng/ml. Exclusion criteria were - medical illness or medications known to affect calcium absorption. Calcium absorption was measured at baseline and after 12 months using a single isotope method with 100 mg calcium and 5 microcuries Ca^{45}, serum Ca^{45} was corrected for weight or BMI. Serum 25OHD and 1,25 $(OH)_2$D were measured by immunoassay (Diasorin). We used multivariate regression that included age, dietary calcium intake, weight, baseline and final calcium absorption and serum 25OHD.

128 women completed the study. Mean baseline serum 25OHD was 14.6 ng/ml (39nmol/L) in Caucasians and 11.6 ng/ml in African Americans. In the longitudinal study mean serum 25OHD increased to 41ng/ml, and there was no difference in final serum 25OHD between groups. There was no increase in 12-month calcium absorption compared to baseline on any dose of vitamin D in either Caucasians or African Americans. In the baseline analysis serum 25OHD < 20ng/ml was divided into quartiles. There was no difference in absorption nor in serum 1,25$(OH)_2$ D amongst quartiles (Anova p 0.86,0.12)

Vitamin D did not increase calcium absorption up to a dose of 2400IU daily or mean serum 25OHD of 41ng/ml. No threshold level of serum 25OHD for calcium absorption was found at baseline or in the longitudinal study suggesting that the threshold for decreased calcium absorption occurs at a very low serum 25OHD level < 5ng/ml. The results suggesting that calcium absorption must reach a plateau at very low levels of serum 25OHD<5 ng/ml.

Calcium absorption measurement: Single isotope method was used to measure calcium absorption at baseline visit and at 12 months. After an overnight fast subjects were asked to drink 5 microcuries (µCi) dose of radioactive calcium (^{45}Ca) in 100 mg of elemental calcium (calcium chloride) that made up to a total volume of 100ml of distilled water. Two hours later, 10 ml of venous blood was collected for analysis of ^{45}Ca. In duplicate samples of 2 ml of serum and 18 ml of scintillation liquid, radiocalcium absorption was measured using a1900 CA Tri-Carb Liquid Scintillation Counter (Packard Instrument, Meriden, CT, U.S.A.) at Creighton University Bone Metabolism Laboratory. Liquid Counter is calibrated every 6 months by the manufacturer.

Results A total of 198 subjects were randomized in the study, 70 subjects withdrew after randomization and 128 subjects (65%) completed the study. The baseline characteristics for the complete study population are separated by dose or by ethnic group in the Tables .

Table 15

		All (n=198)		Placebo (n=38)		400 IU (n=37)		800 IU (n=42)		1600 IU (n=41)		
	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Me
Age y	198	37	6	36	6	36	7	38	6	36	6	
BMI	198	30	7	31	7	31	8	31	6	28	5	
Weight (kg)	198	82	19	85	20	80	21	83	16	79	18	
Serum Calcium mg/dl	197	9.17	0.31	9.09	0.34	9.22	0.29	9.23	0.33	9.17	0.28	9
24hr Urine Calcium mg	197	142	83	138	91	145	73	123	80	159	82	1
Diet Calcium mg	198	655	262	677	275	627	248	599	249	681	282	6
Diet vitamin D IU	198	100	74	108	82	102	69	89	75	113	84	
25OHD ng/ml	198	13.4	4.5	12.7	4.1	13.1	4.2	13.8	4.3	13.3	5.1	1
1,25OHD pg/ml	126	47.6	17.3	42.1	17.1	51.1	18.5	50.5	19.9	50.2	13.9	4
Calcium absorption_%AD/L	126	2.4	1.5	2.1	0.7	2.4	0.6	2.1	0.7	3.1	2.8	
Calcium % absorbed	126	63.0	38.6	57.6	20.6	63.5	17.1	58.1	19.7	76.4	72.5	5
Ca_per_absorbed	126	57.5	21.4	51.6	10.9	57.2	8.2	53.8	9.2	66.9	39.6	5

Table 16

	African Americans			Caucasians			
	n	mean	SD	n	mean	SD	p-value
Age y	79	35	6	119	38	6	0.0019
BMI	79	32	7	119	29	6	<0.0001
Weight (kg)	79	87	20	119	79	17	0.0035
Serum Calcium mg/dl	79	9.1	0.3	118	9.2	0.3	0.23
24hr Urine Calcium mg	78	119.5	79.6	119	157.0	81.5	0.0017
Diet Calcium mg	79	502.8	159.2	119	755.9	268.2	<0.0001
Diet vitamin D	79	96	72	119	103	79	0.22
25OHD ng/ml	79	11.6	4.1	119	14.6	4.4	<0.0001
1,25OHD pg/ml	39	52.2	18.9	87	45.6	16.3	0.045
Calcium absorption %AD/L	37	2.1	0.6	89	2.5	1.7	0.056
Calcium %_absorbed weight corrected	37	60.1	18.8	89	64.2	44.4	0.59

Calcium %_absorbed	37	53.7	8.3	89	59.1	24.8	0.073

There were no significant differences between the groups at baseline. Baseline characters differed by race. The mean (±SD) age of African American women was 35(+6) years and in Caucasian women was 38(+6). The range was 25-45 years. The mean daily intake of calcium from food and supplements was 502.8(+159.2) mg in African American and 755.9 (268.2) in Caucasian women and mean vitamin D intake was 96 vs 103 IU /d respectively.

Effect of Dose on absorption. ***Outcome: 12 month calcium percent absorption***

A multivariate analysis of 12 month calcium percent absorption was conducted looking at race, age, tertile of dietary Calcium, total Calcium, baseline weight, baseline Ca absorption, baseline serum 1,25OHD, baseline serum 25OHD (ng/ml), and dose as predictors in the model.
Dose was not a significant predictor of 12 month Calcium percent absorption. Race was marginally predictive with African Americans tending to have lower 12 month Calcium absorption than Caucasians. Total calcium intake was predictive with a one mg increase in total calcium resulting in a -0.018 decrease in 12 month Calcium percent absorption (p=0.0088). Baseline weight was predictive of Calcium percent absorption with a 1 kg increase in weight resulting in a -0.12 decrease in 12 month Calcium absorption (p=0.019). Baseline Calcium percent absorption also correlates with 12 month Calcium percent absorption. A 1 unit increase in baseline Calcium absorption results in a 0.11 unit increase in 12 month Calcium percent absorption (p=0.012). Adjusted R^2 0.25.

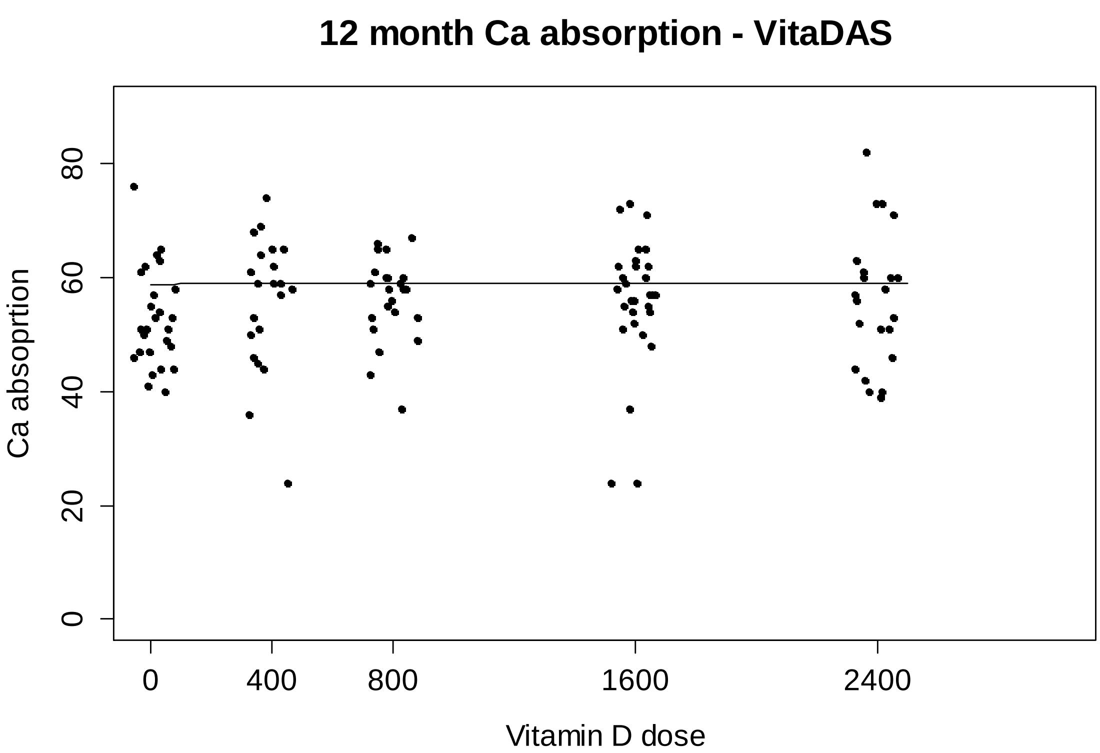
Table 17. Multiple Regression model of 12-month calcium absorption with vitamin D dose as a predictor.

Effect		Estimate	SE	p-value
Intercept		90.648	11.431	<.0001
Race	African America vs. Caucasian	-4.819	2.462	0.053
Age	1 year increase	-0.275	0.159	0.086
Tertile of dietary Calcium mg	216-510.5	-2.145	2.627	0.42
	510.5-700	-0.505	2.316	0.83
	>700	Ref.	-	-
Total Calcium intake	1 mg increase	-0.018	0.007	0.0088

Baseline weight (kg)	1 kg increase	-0.122	0.051	0.019
Baseline Calcium percent absorption	1 unit increase	0.107	0.042	0.012
Baseline serum 1,25OHD pg/ml		-0.045	0.053	0.40
Baseline serum 25OHD (ng/ml)	1 ng/ml increase	0.034	0.228	0.88
Dose	1000 unit increase	0.055	1.026	0.96

Figure 1 shows the dose response effect on 12 month Calcium absorption. These are the results of the multivariate model. Average values were used for the covariates in the model in order to plot the response of dose. We used Caucasian, age=37, dietary Ca>700, total Ca of 891, weight=82, baseline ca absorption of 57.5, baseline serum 1,25OHD=47.6, and baseline serum 25OHD=13.4 in order to plot the model.

Figure 14 . **Vitamin d Dose and 12 month Calcium percent absorption.**



Outcome: 12 month weight corrected calcium absorption
A multivariate analysis of **weight corrected 12 month calcium absorption** (% AD/liter), was conducted looking at race, age, Tertile of dietary Calcium, total Calcium, weight corrected

baseline Calcium absorption (% AD/liter), baseline serum 1,25OHD, baseline serum 25OHD (ng/ml), and dose as predictors in the model.
Dose was not a significant predictor of weight corrected 12 month Calcium absorption. Total calcium was predictive with a one unit increase in total calcium resulting in a -0.034 decrease in weight corrected 12 month Calcium percent absorption (p=0.0097). Weight corrected baseline Calcium absorption also correlates with weight corrected 12 month Calcium absorption. A 1 unit increase in weight corrected baseline Calcium absorption results in a 0.13 unit increase in weight corrected 12 month Calcium percent absorption (p=0.0042).
The adjusted R^2 for this model is 0.15.

Table 18. Multivariate predictors of 15% weight adjusted 12 month calcium absorption (% AD/liter), including dose

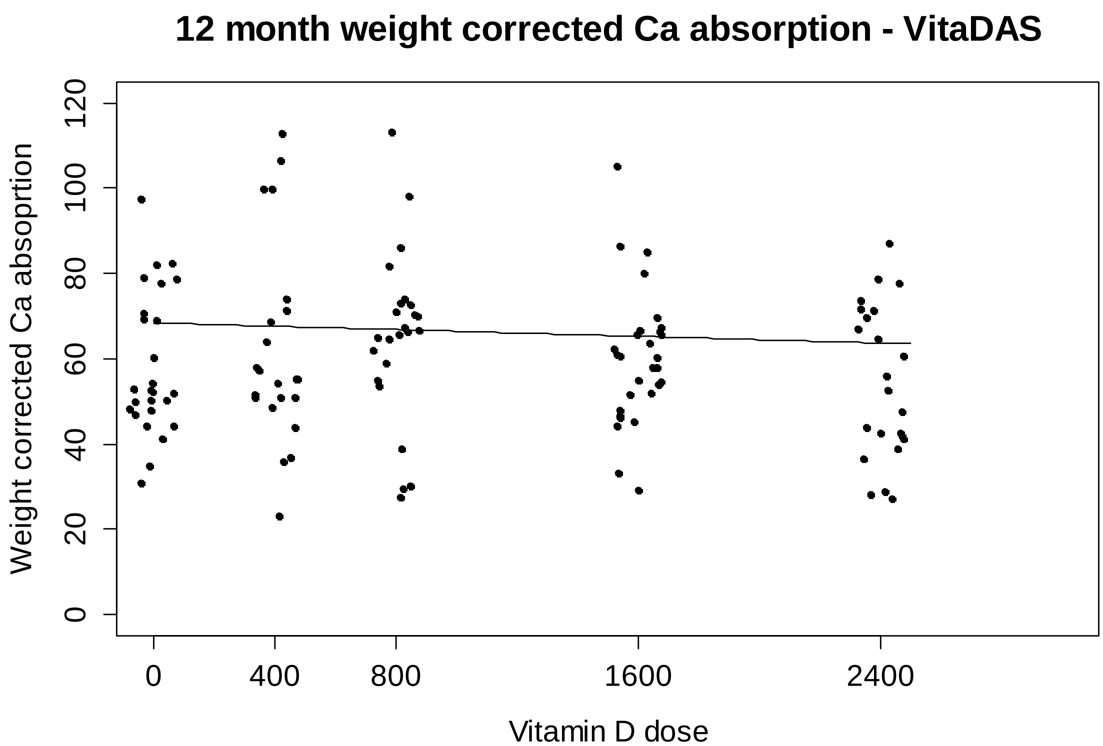
Effect		Estimate	SE	p-value
Intercept		109.4734	19.5590	<.0001
Race	African America vs. Caucasian	-3.8262	4.7518	0.42
Age	1 year increase	-0.3541	0.3097	0.26
Tertile of dietary Calcium	216-510.5	-4.5220	5.0897	0.38
	510.5-700	-2.8945	4.5069	0.52
	>700	Ref.	-	-
Total Calcium mg	1 unit increase	-0.0343	0.0130	0.0097
Weight corrected baseline Ca absorption	1 unit increase	0.1282	0.0438	0.0042
Baseline serum 1,25OHD		0.0280	0.1030	0.79
Baseline serum 25OHD (ng/ml)	1 ng/ml increase	-0.5072	0.4345	0.25
Dose	1000 unit increase	-1.9731	1.9581	0.32

Vitamin D dose was divided by 1000 to fit the models. To estimate the outcome variable, use dose 0 in the models above to correspond to placebo; 0.4 for vitamin D 400 U/d; 0.8 for vitamin D 800 IU/d; 1.6 for vitamin D 1600 IU/d; 2.4 for vitamin D 2400 IU/d

Figure shows the dose response curve for the 12 month weight corrected Calcium absorption. These are the results of the multivariate model. Average values were used for the covariates in the model in order to plot the response of dose. We used Caucasian, age=37, dietary

Calcium>700, total Calcium of 891, baseline weight corrected calcium absorption of 63, baseline serum 1,25OHD=47.6, and baseline serum 25OHD=13.4 in order to plot the model.

Figure 15



Calcium absorption response versus 12 month serum 25OHD level :*Outcome: 12 month calcium percent absorption*

A multivariate analysis of 12 month calcium percent absorption was conducted looking at race, age, tertile of dietary Calcium, total Calcium, baseline weight, baseline Ca absorption, baseline serum 1,25OHD, baseline serum 25OHD (ng/ml), and 12 month serum 25OHD (ng/ml) as predictors in the model.

12 month serum 25OHD was not a significant predictor of 12 month Ca percent absorption. Race was marginally predictive with African Americans tending to have lower 12 month Ca percent absorption than Caucasians. Total calcium was predictive with a one unit increase in total calcium resulting in a -0.018 decrease in 12 month Ca percent absorption ($p=0.011$). Baseline weight was predictive of Ca percent absorption with a 1 kg increase in weight resulting in a -0.12 decrease in 12 month Ca percent absorption ($p=0.018$). Baseline Ca percent absorption also correlates with 12 month Ca percent absorption. A 1 unit increase in baseline

Ca percent absorption results in a 0.11 unit increase in 12 month Ca percent absorption (p=0.011). adjusted R^2 for this model is 0.25.

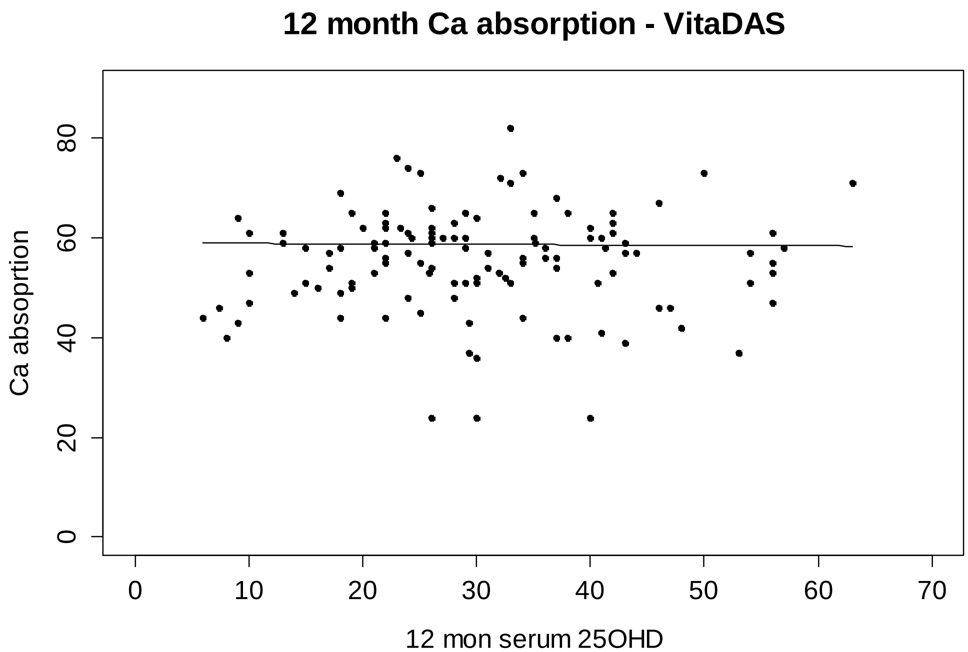
Table 19. Multivariate predictors of 12 month calcium percent absorption, including serum 25OHD- Vitadas

Effect		Estimate	SE	p-value
Intercept		90.860	11.419	<.0001
Race	African America vs. Caucasian	-4.842	2.443	0.050
Age	1 year increase	-0.276	0.159	0.085
Tertile of dietary Ca	216-510.5	-2.134	2.609	0.42
	510.5-700	-0.499	2.312	0.83
	>700	Ref.	-	-
Total Ca	1 unit increase	-0.018	0.007	0.011
Baseline weight (kg)	1 kg increase	-0.124	0.051	0.018
Baseline Ca percent absorption	1 unit increase	0.107	0.042	0.011
Baseline serum 1,25OHD		-0.044	0.053	0.41
Baseline serum 25OHD (ng/ml)	1 ng/ml increase	0.039	0.231	0.87
12 month serum 25OHD (ng/ml)	1 ng/ml increase	-0.010	0.076	0.90

Figure 16 shows the 12 month serum 25OHD as a predictor of 12 month Calcium percent absorption. These are the results of the multivariate model. Average values were used for the

covariates in the model in order to plot the response of dose. We used Caucasian, age=37, dietary Calcium>700, total Calcium of 891, weight=82, baseline calcium absorption of 57.5, baseline serum 1,25OHD=47.6, and baseline serum 25OHD=13.4 in order to plot the model.

Figure 16



12 month serum 25OHD was not a significant predictor of 12 month Calcium absorption. Total calcium was predictive with a one unit increase in total calcium resulting in a -0.0011 decrease in 12 month Calcium absorption (p=0.0098). Baseline weight was predictive of Calcium absorption with a 1 kg increase in weight resulting in a -0.007 decrease in 12 month Calcium absorption (p=0.029). Baseline Calcium absorption also correlates with 12 month Calcium absorption. A 1 unit increase in baseline Calcium absorption results in a 0.10 unit increase in 12 month Calcium absorption (p=0.0082).

The adjusted R^2 for this model is 0.20.

Table 20. Multivariate predictors of 12 month calcium absorption (% AD/liter), including serum 25OHD- Vitadas

Effect		Estimate	SE	p-value
Intercept		4.40044	0.71427	<.0001
Race	African America vs. Caucasian	-0.23265	0.15505	0.14
Age	1 year increase	-0.01409	0.01008	0.17
Tertile of dietary Ca	216-510.5	-0.09352	0.16539	0.57
	510.5-700	-0.06220	0.14645	0.67
	>700	Ref.	-	-
Total Ca	1 unit increase	-0.00114	0.00043	0.0098
Baseline weight (kg)	1 kg increase	-0.00720	0.00325	0.029
Baseline Ca absorption (% AD/liter)	1 unit increase	0.10087	0.03744	0.0082
Baseline serum 1,25OHD		-0.00032	0.00338	0.92
Baseline serum 25OHD (ng/ml)	1 ng/ml increase	-0.00187	0.01465	0.90
12 month serum 25OHD (ng/ml)	1 ng/ml increase	0.00001	0.00485	1.0

Outcome: 12 month weight corrected calcium absorption

A multivariate analysis of **weight corrected 12 month calcium absorption (% AD/liter)** was conducted looking at race, age, tertile of dietary Calcium, total Calcium, weight corrected baseline Calcium absorption (% AD/liter), baseline serum 1,25OHD, baseline serum 25OHD (ng/ml), and 12 month serum 25OHD (ng/ml) as predictors in the model.

12 month serum 25OHD was not a significant predictor of weight corrected 12 month Calcium absorption. Total calcium was predictive with a one unit increase in total calcium resulting in a -0.033 decrease in weight corrected 12 month Calcium absorption (p=0.015). Weight corrected baseline Calcium absorption also correlates with weight corrected 12 month Calcium absorption. A 1 unit increase in weight corrected baseline Calcium absorption results in a 0.12 unit increase in weight corrected 12 month Calcium absorption (p=0.0058). The adjusted R^2 for this model is 0.15.

Figure 17 12month on the weight corrected absorption

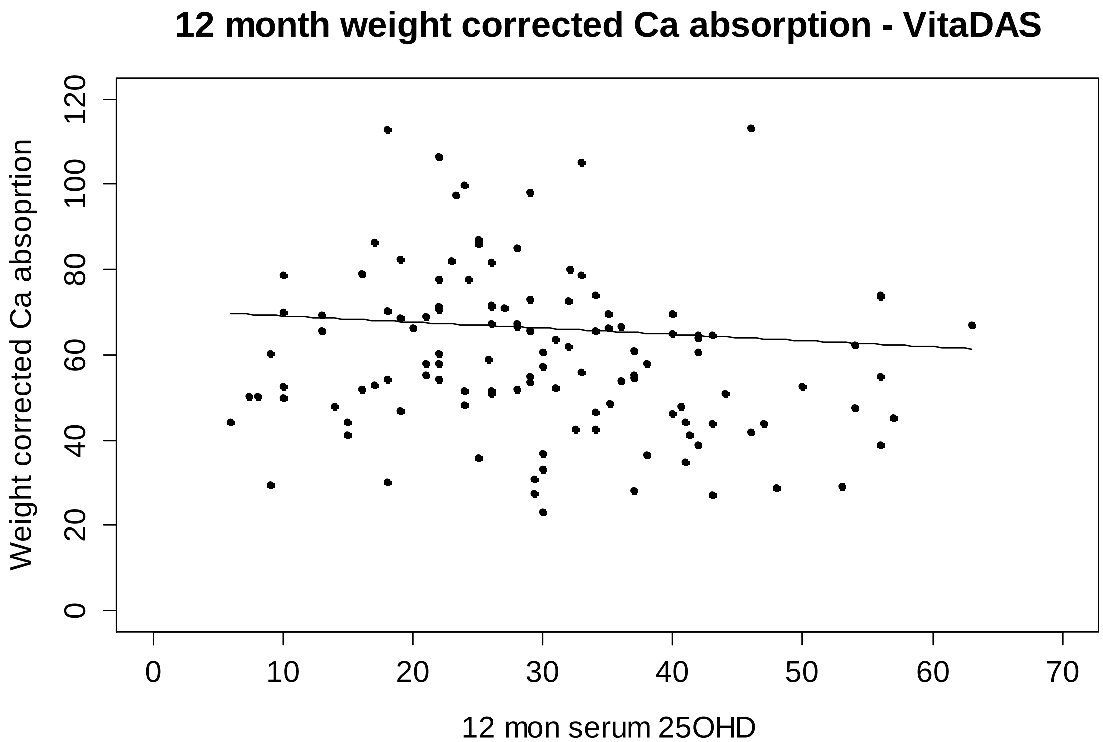
Table 21. Multivariate predictors of weight corrected 12 month calcium absorption (% AD/liter), including serum 25OHD- Vitadas

Effect		Estimate	SE	p-value
Intercept		108.838	19.547	<.0001
Race	African America vs. Caucasian	-3.328	4.697	0.48
Age	1 year increase	-0.351	0.310	0.26
Tertile of dietary Ca	216-510.5	-5.226	5.031	0.30
	510.5-700	-3.180	4.492	0.48
	>700	Ref.	-	-
Total Ca	1 unit increase	-0.033	0.013	0.015
Baseline weight corrected Ca absorption	1 unit increase	0.123	0.044	0.0058
Baseline serum 1,25OHD		0.038	0.104	0.71
Baseline serum 25OHD (ng/ml)	1 ng/ml increase	-0.402	0.445	0.37
12 month serum 25OHD (ng/ml)	1 ng/ml increase	-0.146	0.146	0.32

Figure 4 shows the 12 month serum 25OHD as a predictor of 12 month weight corrected Calcium absorption. These are the results of the multivariate model. Average values were used for the covariates in the model in order to plot the response of dose. We used Caucasian, age=37, dietary Calcium>700, total Calcium of 891, baseline weight corrected calcium absorption of 63, baseline serum 1,25OHD=47.6, and baseline serum 25OHD=13.4 in order to plot the model.

Outcome: 12 month calcium absorption (%AD/Liter)

A multivariate analysis of **12 month calcium absorption (% AD/liter)** was conducted looking at race, age, tertile of dietary Calcium, total Calcium, baseline weight, baseline Calcium absorption (% AD/liter), baseline serum 1,25OHD, baseline serum 25OHD (ng/ml), and 12 month serum 25OHD (ng/ml) as predictors in the model.



Since the African American and Caucasian women had difference between body weights, which was a significant predictor of calcium absorption (p=0.0035), it was adjusted by 15%. After weight correction we found that the calcium absorption levels at baseline were almost same in both the groups despite having significant difference in the 1,25(OH) D levels (p=0.045).

We looked for any threshold change in calcium absorption according to the baseline serum 25OHD values. When the baseline of serum 25OHD levels were divided into 4 groups of 0-5, 5-10,11-15 and 16-20ng/ml, there was no difference in absorption nor in serum 1,25$(OH)_2$D levels amongst serum 25OHD groups (ANOVA p 0.86,0.12).

TASK 3 (ii) Effect of Vitamin D on bone markers and bone mineral density

This task is completed

Statistical analysis T-tests and analysis of variance (Anova) were used to look at the effect of race and dose on BMD and bone markers. P-values less than 0.05 are considered to be statistically significant. SAS software was used for data analysis.

Results

198 women were randomized to dose group, 79 African Americans and 119 Caucasians. Table 2 shows Bone mineral density (BMD) and bone resorption marker information by race. T-tests show that African American and Caucasian women BMD measurements are significantly different at baseline and 12 months as expected since it is well known that African Americans have higher bone mass, however the percentage change in BMD after treatment does not differ by race.

Table 22. Baseline BMD and bone markers by race

	All			African American			Caucasian			
	N	Mean	SD	N	Mean	SD	N	Mean	SD	p-value
Baseline Femoral Neck BMD	**126**	**0.88**	**0.13**	**38**	**0.97**	**0.11**	**88**	**0.84**	**0.12**	**<0.0001**
12 month Femoral Neck BMD	127	0.89	0.13	39	0.97	0.11	88	0.85	0.12	<0.0001
% change Femoral Neck BMD	126	0.79%	3.02%	38	0.99%	2.97%	88	0.70%	3.05%	0.62
Baseline Total body BMD	**126**	**1.00**	**0.13**	**38**	**1.06**	**0.12**	**88**	**0.97**	**0.13**	**0.0009**
12 month Total body BMD	127	1.01	0.14	39	1.06	0.12	88	0.98	0.14	0.0011
% change Total body BMD	126	0.57%	2.09%	38	0.46%	1.90%	88	0.62%	2.17%	0.70
Baseline Spine BMD	**127**	**1.10**	**0.13**	**39**	**1.17**	**0.13**	**88**	**1.06**	**0.11**	**<0.0001**
12 month Spine BMD	127	1.11	0.13	39	1.19	0.14	88	1.07	0.11	<0.0001
% change Spine BMD	127	1.22%	2.41%	39	1.69%	2.52%	88	1.01%	2.34%	0.14
Baseline Bone marker	**124**	**15.60**	**5.07**	**35**	**16.68**	**5.31**	**89**	**15.17**	**4.94**	**0.14**
12 month Bone marker	124	15.51	5.38	35	15.76	5.52	89	15.42	5.35	0.75
% change Bone marker	124	1.05%	23.03%	35	-3.51%	22.79%	89	2.84%	23.01%	0.17

Efffect of dose on the percent change in BMD and bone markers

In order to maximize numbers the vitamin D dose was combined into 3 groups, placebo, 400-800 IU(low dose) and 1600-2400 IU (medium dose). ANOVA models were used to look at the effect of race and dose group on percent change in BMD and bone markers. Included in the model were main effects for race, dose and the interaction between race and dose. For each of the models the interaction between race and dose was not statistically significant indicating that the effect of dose on outcome was similar in both races. We did not find a significant difference in percent change in BMD by dose in any of the models.

Table 23 Effect of race and dose group on BMD and bone markers

	Race	Dose group	Mean	SE	Interaction p-value	Dose p-value
% change Femoral Neck BMD	African American	Placebo	0.53	1.01	0.66	0.49
	Caucasian	Placebo	1.26	0.70		
	African American	400-800	1.61	0.79		
	Caucasian	400-800	0.92	0.56		
	African American	1600-2400	0.63	0.81		
	Caucasian	1600-2400	0.28	0.48		
% change Total body BMD	African American	Placebo	0.15	0.70	0.95	0.33
	Caucasian	Placebo	0.14	0.48		
	African American	400-800	0.75	0.54		
	Caucasian	400-800	1.09	0.39		
	African American	1600-2400	0.35	0.56		
	Caucasian	1600-2400	0.51	0.33		
% change Spine BMD	African American	Placebo	2.04	0.81	0.85	0.56
	Caucasian	Placebo	0.90	0.55		
	African American	400-800	1.41	0.60		
	Caucasian	400-800	0.63	0.45		
	African American	1600-2400	1.78	0.65		
	Caucasian	1600-2400	1.34	0.38		
% change Bone marker	African American	Placebo	5.46	8.08	0.19	0.41
	Caucasian	Placebo	4.63	5.24		
	African American	400-800	-2.06	6.11		
	Caucasian	400-800	-2.42	4.17		
	African American	1600-2400	-10.58	6.34		
	Caucasian	1600-2400	5.93	3.61		

Task 5____(1) Adverse Events. **(2) Biochemical changes in serum/urine calcium.**
This task is completed

Serious adverse events (SAE) are listed by subject number. There have been five serious adverse events in four subjects that were not related to the study drug.

Table 24. Serious Adverse Events

bject mber	Dose	Description of SAE	Date of Occurrence	Date of Cessation	Intensity	Relationship to Study drug	Baseline visit date	Drop D
208	800	Internal bruising/ bleeding (due to auto accident)	07/22/2008	07/25/2008	Moderate	Definitely Not	05/19/2008	Comple d study 5/18/09
270	400	Subarachnoid hemorrhage	03/10/2010	03/17/2010	Life Threatening	Definitely Not	5/18/2009	4/20/20
270	400	Fronto-temporal craniotomy/ resection of hemangioma	03/17/2010	03/17/2010	Life Threatening	Definitely Not	5/18/2009	4/20/20
534	800	Maxillary Hypoplasia surgery	04/21/2011	04/21/2011	Severe	Definitely Not	6/4/2010	Comple d study 7/6/201
537	400	Broken R ankle + tibia	02/02/2012	02/02/2011	Severe	Definitely Not	8/10/2010	Comple d study 8/15/20

None of these events can be attributed to the interventions
All other adverse events are listed in the table in the Appendix

Serum & Urine Calcium Results

Serum calcium results are available for 198 subjects at baseline, 165 subjects at 3 months, 143 subjects at 6 months, 133 at 9 months, and 124 subjects at 12 months. Elevated serum calcium is defined as fasting serum calcium >10.6 mg/dl or >0.3 mg/dl above the upper limit of normal (>=10.3 mg/dl). If the serum calcium is elevated then the test was repeated 2 weeks later. Two subjects had elevated serum calcium, the test was not repeated for subject 60432 because the criteria for hypercalcemia was 10.6 during treatment. (see Table 15)

Urine calcium results are available for 198 subjects at baseline, 157 subjects at 3 months, 135 subjects at 6 months, 131 at 9 months, and 120 subjects at 12 months. Elevated urine calcium is defined as 24 hour urine calcium >290 mg at baseline or >400 mg at any of the other visits. Table 15 shows the subjects who had elevated urine calcium measurements at any visit and the repeated measurements. Seven (3.5%) subjects had elevated urine calcium at baseline, 1 (0.6%) subject had elevated at the 3 month visit, 1 (0.7%) subject was elevated at the 6 month visit, 1

(0.8%) subject was elevated at the 9 month visit, and 0 (0%) subjects had elevated urine calcium at the 12 month visit. Subject number 60004 was discontinued because she refused to retest at the 9-month visit.

Table 25. Subjects with elevated 24 hour urine calcium (>290 mg at baseline, and >400 mg at other visits) or serum calcium

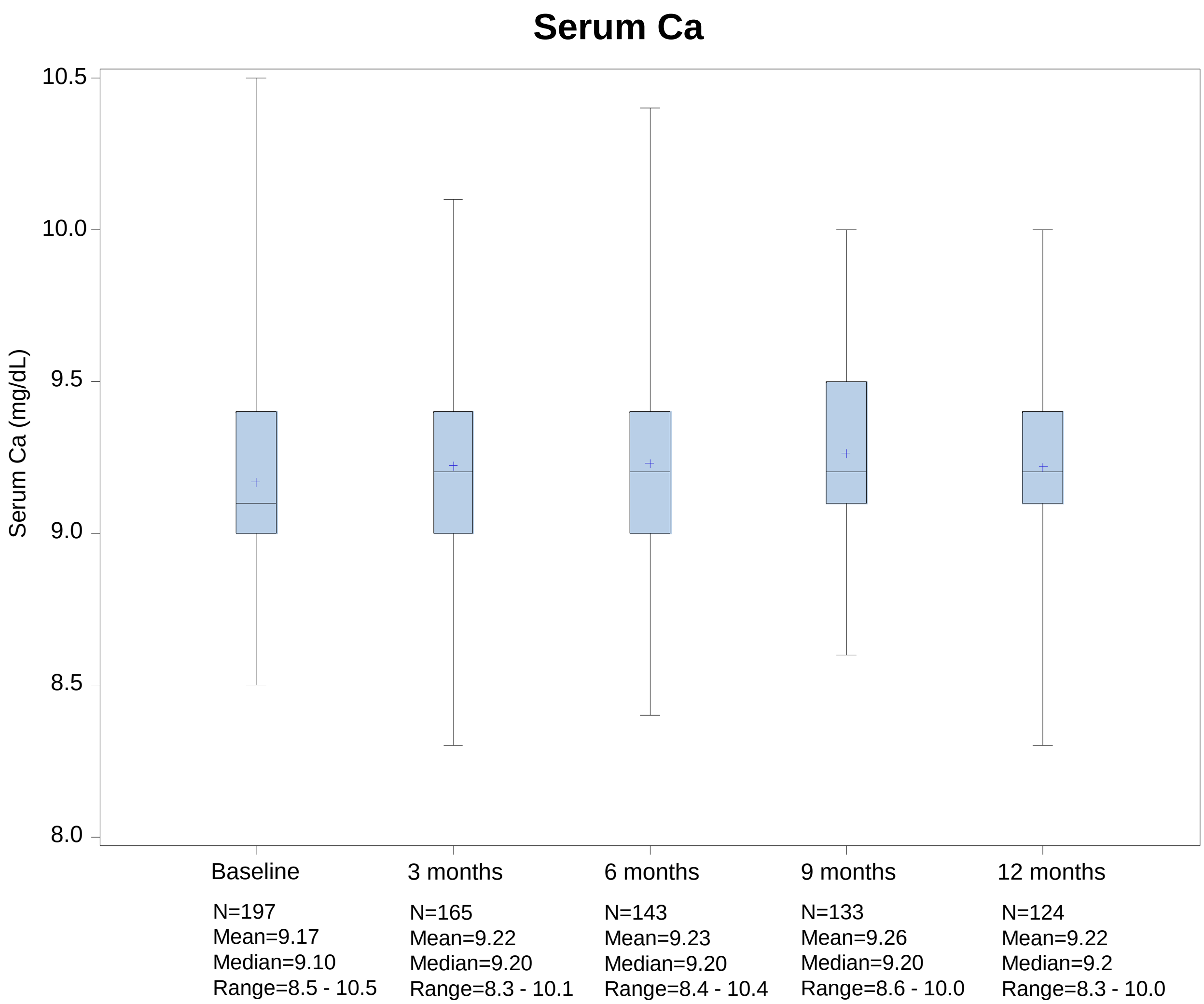
Subject Number	race	dose	Urine Calcium										Serum Ca			
			Baseline			3 month			6 month		9 month	12 month	Baseline		3 mon	n
			1st	2nd	3rd	1st	2nd	3rd	1st	2nd	1st	1st	1st	2nd	1st	
60004	C	2400	164			260			183		510*		9.3		9.2	
60040	C	400	287			411	408	246	251		211	139	9.4		9.3	
60042	C	800	48						73		77	62	10.5	9.4	9.5	
60055	C	0	298	161		182			190		259	261	9.3		9.2	
60212	C	2400	319	154		204			222		357	210	9.3		9.5	
60220	C	1600	319	286		274			389		243	220	9		8.9	
60248	C	2400	329	227		218			190		307	357	9.1		9.1	
60249	C	0	359	250		217			245		204	218	9.1		8.8	
60432	AA	400	112			101					53	34	9.8		9.4	1
60612	AA	800	375	399	296	160			150		170	85	9.4		9.6	
60619	AA	2400	335	143		340			370							
60625	AA	400	251			396			480	326	230	293	9.1		9.3	

* Patient refused retest, dropped from the study.

The following Figures 14,15,16 show boxplots of the serum and urine calcium levels at the 5 visits, combining dose levels and races. As per protocol several of the measurements had to be repeated. If multiple measurements were done, then the first level was used for the following analysis (usually the highest). The boxplot shows the distribution of the data. The line in the middle of the box is the median (50th percentile), the plus symbol is the mean, the lower edge of the box is the 1st quartile (25th percentile), the upper edge of the box is the 3rd quartile (75th percentile), and the lines extending from the box (called whiskers) show the minimum and maximum of the data.

From the boxplot, figure 14 we can see the distribution of serum calcium levels. None had serum calcium levels greater than 10.6 mg/dl.

Figure 18. Serum calcium data.



From the boxplot, Figure 15, we can see the distribution of the 24 hour urine calcium levels. 7 (3.5%) subjects had elevated urine calcium at baseline, 1 (0.6%) subject had elevated at the 3 month visit, 1 (0.7%) subject was elevated at the 6 month visit, 1 (0.8%) subject was elevated at the 9 month visit, and 0 (0%) subjects had elevated urine calcium at the 12 month visit.

Figure 19. 24h Urine calcium data

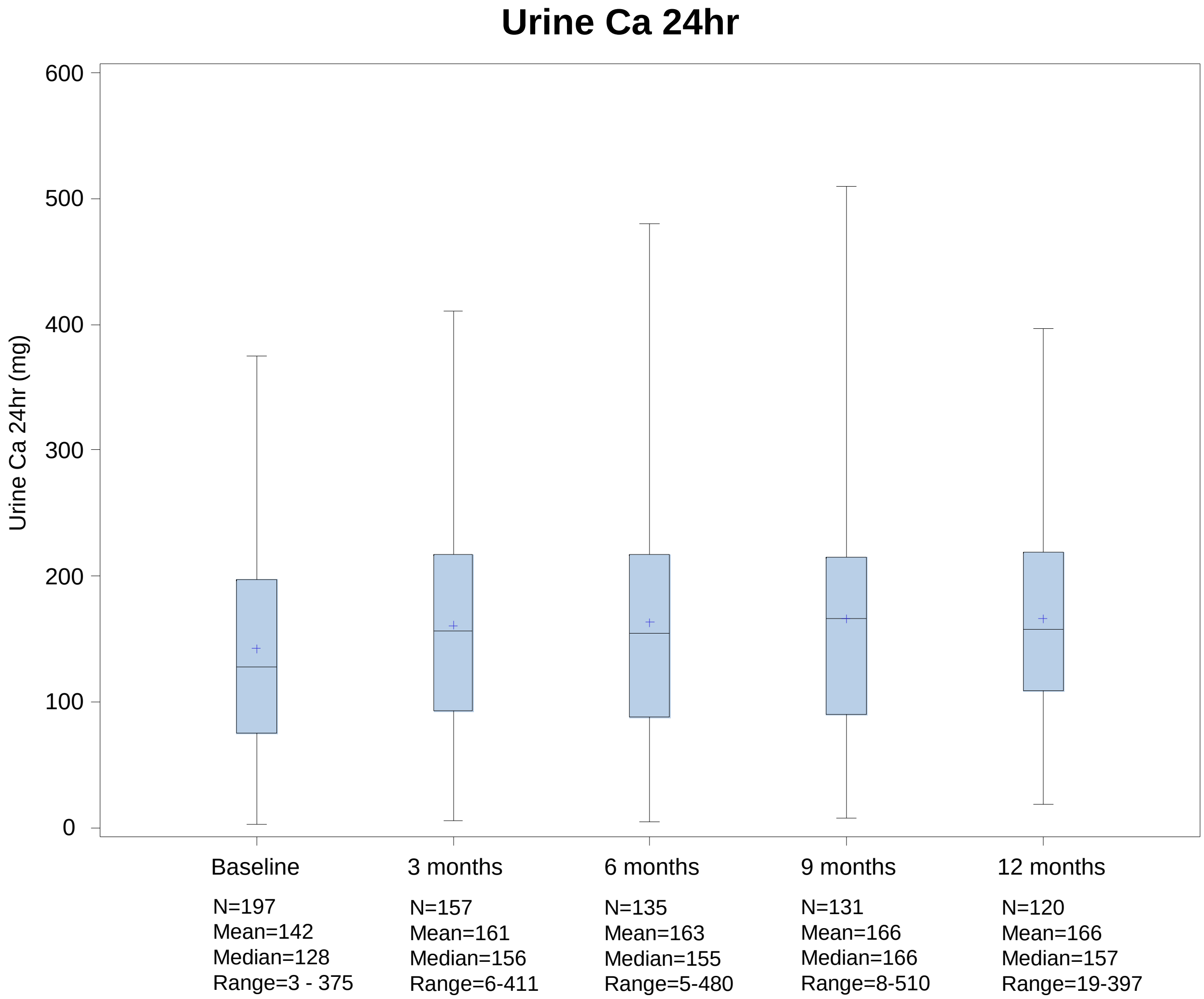
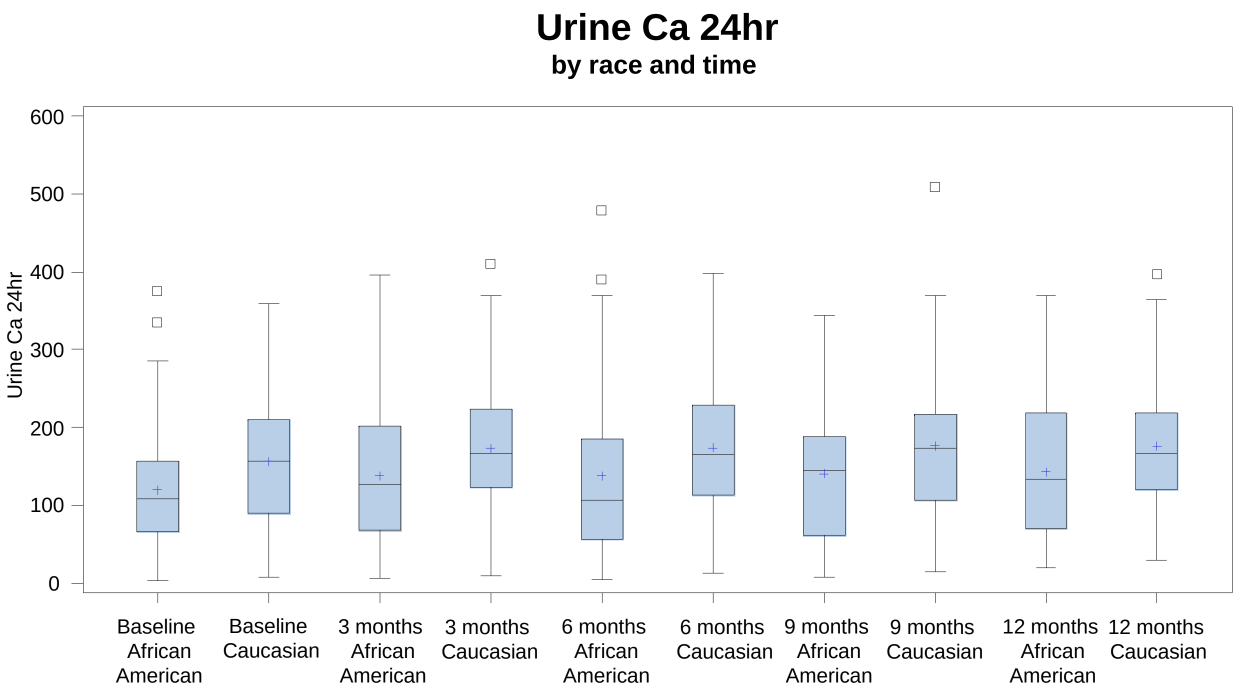


Figure 20. 24 h Urine calcium data by race and visit



N	78	119	55	102	42	93	38	93	34	86
Mean	119.5385	157.0252	137.9636	173.1471	138.4762	174.1398	140.5	177.0538	142.7647	175.593
Std Dev	79.61314	81.53594	90.61436	80.45642	105.4464	87.81572	94.15305	88.39528	89.17333	79.34398
Min	3	7	6	10	5	12	8	14	19	29
Max	375	359	396	411	480	397	344	510	369	397

24h urine calcium is significantly lower in African American at baseline by 37mg and although both groups increase 24h urine calcium on vitamin d and calcium ,urine calcium is always lower by about the same amount compared to Caucasians.

Model of serum PTH and serum 25OHD

We examined the model looking at PTH with race and serum 25OHD as predictors. Serum 25OHD is measured at the same time point as PTH in the model. There was a significant race by serum 25OHD interaction, therefore, the races were modeled separately. In the mixed model, serum 25OHD is a significant predictor of serum PTH. As serum 25OHD increases $\log_{10}$ (PTH) decreases. See table 16 and Figures 17 and 18.

Table 26. Dose response mixed effects model, estimating the dose response of $\log_{10}$(PTH) at each time point – all doses.

		African American					Caucasian				
				95% confidence interval					95% confidence interval		
Effect		β estimate	SE	Lower limit	Upper limit	Overall P-value	β estimate	SE	Lower limit	Upper limit	Overall P-value
Intercept		1.6285	0.02574	1.5773	1.6798		1.5243	0.0176	1.4895	1.5592	
Serum 25OHD	1 ng/ml increase	-0.00509	0.001026	-0.00713	-0.00305	<0.0001	- 0.00284	0.000551	- 0.00393	- 0.00175	<0.0001

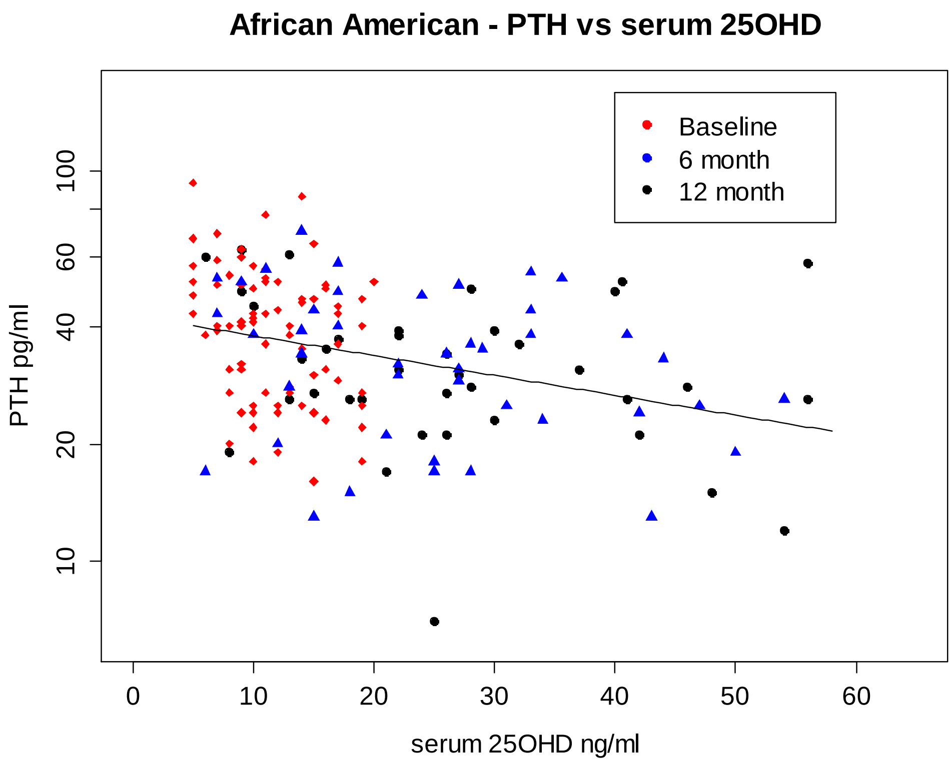
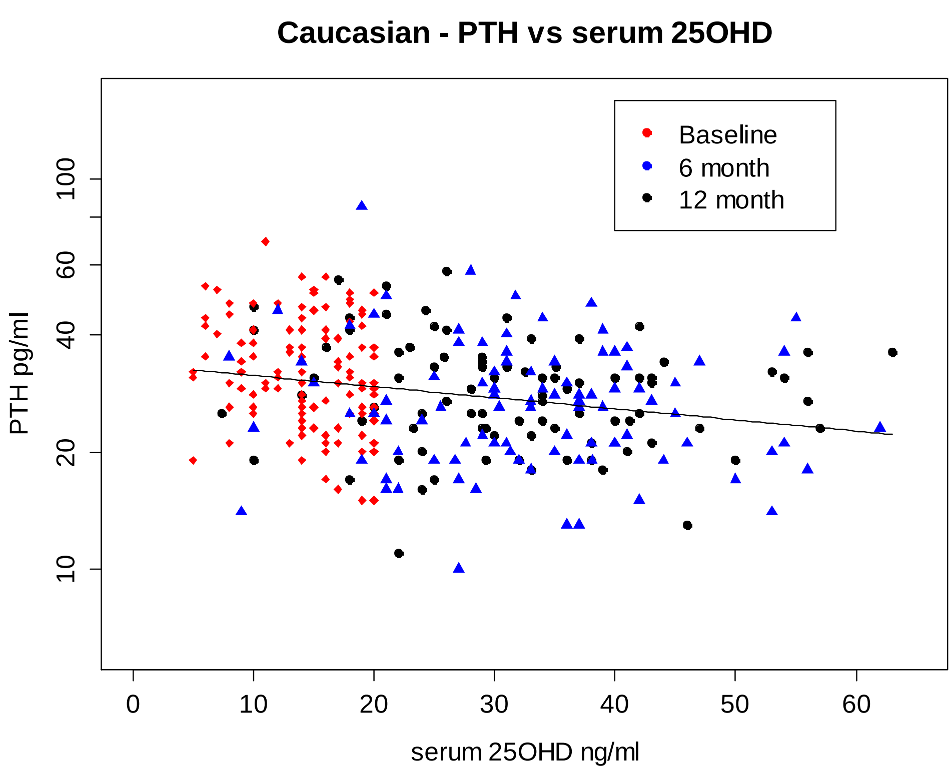


Figure 22. PTH and serum 25OHD in Caucasian women



In conclusion, serum PTH is significantly related to serum 25OHD but not the vitamin d dose probably because of the large individual variation in response to a dose.

KEY RESEARCH ACCOMPLISHMENTS

Tasks 1and 2 and 4

- Established for the first time the RDA and EAR for vitamin D in younger Caucasian and African American women. The RDA for young Caucasian women is 400IU and for African Americans is between 800-1600 IU based on a serum 25OHD level of 20ng/ml, however this finding may have occurred by chance.
- the response in serum 25OHD to vitamin D was slightly greater in African Americans it was because they started with lower vitamin d levels.
- Serum parathyroid hormone decrease on vitamin D treatment. The significance of this result in young people is unclear although in older people it reduces bone loss.

Task 3 and 4

- There was no significant effect of vitamin D on calcium absorption. Calcium absorption was slightly but not significantly lower in African Americans compared to Caucasians .

Task 5 and 4

- This treatment combination is safe over one year. There were no serious adverse events related to the vitamin D or calcium over one year. However hypercalciuria could be a safety issue long-term if subjects are predisposed to kidney stones (6% of the population). Hypercalciuria may be due to the extra calcium and not the vitamin D dose.

REPORTABLE OUTCOMES

- TASKS 1,2 4 and 5 results have been accepted for publication by 'The Journal of Bone and Mineral Research' (impact factor 6.4)
  These results are all included in the paper-
  Gallagher, JC, Jindal PS, Smith LM. Vitamin D Supplementation in young Caucasian and African American women. J Bone Mineral Res November 2013, will be online in about 2 weeks.

CONCLUSIONS

1] We believe that this study is notable because for the first time it established an RDA for vitamin D in young Caucasian and African American women. This was one of the recommended gaps in research noted by the IOM in the 1997 and 2011 report (2).
The label on the nutritional vitamins bottles is expressed as percent of RDA 400IU daily will represent 100 percent of the RDA.

A similar vitamin D dose study that we performed in 263 older Caucasian and African American women was funded by the NIH showed that the RDA in older women was 800 IU. (8,9,10).

Later this year all data from both studies will be combined for a total of 471 women (189 African American and 282 Caucasian). Our prediction with larger numbers is that the vitamin D dose response will be the same in young and old, Caucasian and African American women-in other words there will be no age effect and no ethnic difference in the response to vitamin D.

Future recommendation is to do a similar comparative study between Caucasian and Asian and Hispanics. At the moment all RDA's for vitamin D are based on Caucasians.

2] there was no effect of vitamin d (upto 2400IU) on calcium absorption

3] no effect of vitamin D on bone density and bone resorption markers

So what

- There is real value from these study results as it relates to future IOM recommendation regarding the RDA for vitamin D. At the time of the IOM report it was noted that there was no data in young people of any ethnicity and the (guestimate) recommendation for the RDA was 400 IU. Our results support this IOM recommendation.
- In the last 2 years testing of vitamin D levels has cost this country about 20 billion dollars. Our study showed that vitamin D 400 IU daily-achieved normal levels in 97.5% and testing would have been unnecessary. Direct costs for this study were $530,000.

- There may be application to DOD members. Our results are in agreement with the IOM recommendations that in the age group 20-50 years vitamin D intake should be 400 IU daily either through the diet or diet + sun exposure. There is some uncertainty about African Americans .It is between 400-1600 IU daily but caution is necessary because the study numbers in this study were small.

- In a study of ~5,200 navy recruits in basic training vitamin D 800 IU plus calcium 2,000mg daily reduced stress fractures by 20 percent (11). However, this study should be repeated with calcium, vitamin D 400IU or vitamin D 400IU + calcium together because we are unsure of the contribution of the calcium to fracture reduction. This study could be simplified by using a single small dose of vitamin D once a week or a larger dose 2-3 months prior to basic training.

- There was no effect of vitamin D intervention on calcium absorption and this result contradicts 'prevailing wisdom' amongst bone and mineral researchers as well as industry that widely markets vitamin D for increasing calcium absorption!

References

1. WHO Scientific Group on the Prevention and Management of Osteoporosis 2003 Prevention and management of osteoporosis: report of a WHO scientific group. Geneva: World Health Organization.

2. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline.J Clin Endocrinol Metab 2011;96:1911-30.

3. Institute of Medicine. Dietary reference intakes for calcium and vitamin D 2011 Washington, DC: The National Academies Press, Available from: http://www.ncbi.nlm.nih.gov/books/NBK56072.

4. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Manson JE, Mayne ST, Ross AC, Shapses SA, Taylor CL. IOM committee members respond to Endocrine Society vitamin D guideline. J Clin Endocrinol Metab. 2012 Apr;97(4):1146-52.

5. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT Vitamin D status: United States(2001–2006). 2011;NCHS data brief, no 59. Hyattsville, MD: National Center for Health Statistics.

6. Park T and Lee SY (1997). A test of missing completely at random for longitudinal data with missing observations. *Statistics in Medicine*, 16, 1859-1871.

7. Fitzmaurice and Laird (2000). Generalized linear mixture models for handling nonignorable dropouts in longitudinal studies. Biostatistics, 1, 2, 141-156.

8. Gallagher JC,Sai Aj,Templin TJ,Smith,LM Dose response to vitamin D supplementation in postmenopausal women 2011 A randomized clinical trial Ann Intern Med. 2012;156:425-437.

9. Gallagher JC, Peacock M, Yalamanchili V, Smith LM. Effects of vitamin d supplementation in older african american women.J Clin Endocrinol Metab. 2013 Mar;98(3):1137-46

10. Gallagher JC, Yalamanchili V, Smith LM. The effect of vitamin D on calcium absorption in older women.J Clin Endocrinol Metab. 2012; 97(10):3550-6.

11. Lappe J, Cullen D, Haynatzki G, Recker R, Ahlf R, Thompson K.Calcium and vitamin d supplementation decreases incidence of stress fractures in female navy recruits. J Bone Miner Res. 2008 May;23(5):741-9.

Appendix
The following table includes all the medical events for the study.

Table Serious Adverse Events (SAE), adverse events and unanticipated problems

Number	Description of Medical Event	Date of Onset	Date of Cessation	Serious?
60001	chest cold	2/2/2009	2/25/2009	No
60003	stomach pain/ulcer worseninq (pre-existinq)	8/22/2008		No
60003	cortisone shot -back pain	10/17/2008	10/17/2008	No
60003	right wrist surgery -cyst removed	10/29/2008	10/29/2008	No
60004	back spasm	10/11/2008	10/11/2008	No
60004	riqht ankle sprain from fall	10/11/2008	11/5/2008	No
60004	right hip bruisinq from fall	10/11/2008	11/5/2008	No
60004	riqht wrist pain/ swellinq from fall	10/11/2008	11/5/2008	No
60004	sinus infection	11/17/2008		No
60005	stomach flu	12/5/2008	12/6/2008	No
60005	stomach flu	3/3/2009	3/6/2009	No
60005	upper respiratory cold/cough	3/3/2009	3/22/2009	No
60005	bronchitis	5/6/2009	5/18/2009	No
60007	hypertension	12/10/2008	6/12/2009	No
60008	back pain	9/14/2008		No
60008	riqht hip pain	9/14/2008		No
60008	head cold	12/14/2008	12/28/2008	No
60008	left knee pain, aching, stiff & sore	3/17/2009		No
60010	hypothyroid	7/1/2009		No
60010	bruised/scraped left foot from tripping	12/5/2009	12/12/2009	No
60013	head cold -cough , stuffed head	2/1/2010	2/14/2010	No
60015	H1N1 flu	10/12/2009	10/16/2009	No
60015	chest cold	10/31/2009	11/6/2009	No
60015	chest cold	2/2/2010		No
60016	sinus infection	3/26/2009	4/2/2009	No
60016	strep throat	2/5/2010		No
60017	UTI	2/9/2009	3/1/2009	No
60017	bruised calves & buttocks	4/1/2009	4/6/2009	No
60017	viral infection -headache fatigue	4/27/2009	6/1/2009	No
60017	dizzy spells	7/5/2009	7/20/2009	No
60017	burns on arms (1st degree)	7/18/2009	7/25/2009	No
60017	bruised buttocks	10/1/2009	10/13/2009	No
60017	cold	10/1/2009	10/1/2009	No
60017	crampinq in leqs	10/1/2009	11/1/2009	No
60018	headaches	10/5/2009		No

60019	sleep apnea (obstructive & central)	2/1/2010		No
60019	bigger appetite	2/23/2009	4/1/2009	No
60019	heavy spotting -patient on Seasonique -period unexpected	6/10/2009	7/1/2009	No
60019	sinus infection	2/3/2010	2/10/2010	No
60020	knee scrape from fall	10/18/2009	10/25/2009	No
60022	moodiness	5/4/2009	12/1/2009	No
60022	reflux worsening (pre-existing)	8/19/2009		No
60022	Cold	12/1/2009	12/1/2009	No
60024	left knee pain poss frayed meniscus	8/3/2009	10/5/2009	No
60024	flu (fatigue and chills)	11/4/2009	11/5/2009	No
60024	nausea/vomiting	11/14/2009	11/15/2009	No
60024	root canal	12/31/2009	12/31/2009	No
60025	slight depression	7/1/2009		No
60026	head cold	8/30/2009	9/7/2009	No
60026	ankle injury	9/1/2009	9/8/2009	No
60026	sinus infection	9/7/2009	9/17/2009	No
60026	tonsillitis	11/5/2009	11/15/2009	No
60027	nausea/vomiting from Ca absorption	2/16/2009	2/16/2009	No
60028	itching	2/20/2009		No
60031	constipation	3/13/2009	3/20/2009	No
60031	scraped knee -riqht leg	8/26/2009	8/30/2009	No
60031	cold	9/4/2009	9/14/2009	No
60031	scraped knee -left leg	12/22/2009	12/27/2009	No
60031	scraped knee -left leg	1/17/2010	1/23/2010	No
60031	outpatient surgery to remove polyps on vocal cords	3/9/2010	3/9/2010	No
60032	head cold	3/1/2009	3/1/2009	No
60032	hair loss	6/1/2009		No
60033	hives after calcium absorption-went away	2/20/2009	2/20/2009	No
60033	ovarian cyst	6/3/2009		No
60038	upper respiratory infection	3/30/2009	4/3/2009	No
60038	allergic reaction to bee sting	9/27/2009		No
60040	decreased eye siqht -noticed x 6 months	1/1/2009		No
60040	granuloma annulare	6/5/2009		No
60040	heavy menstrual bleeding	12/4/2009	12/24/2009	No
60040	uterine ablation	2/4/2010	2/4/2010	No
60040	uterine ablation pain	2/4/2010	2/4/2010	No
60041	diarrhea/gassy	6/1/2009	1/1/2010	No
60041	gastroparesis	10/1/2009		No
60041	anemic	2/1/2010		No
60042	hot flashes	9/18/2009		No
60042	sinus congestion	1/30/2010	2/1/2010	No

60045	1 floater in OD	4/17/2009		No
60046	yeast infection	7/12/2009	7/26/2009	No
60046	infected finger from splinter	1/1/2010	2/1/2010	No
60046	riqht elbow sore	3/1/2010		No
60047	benign lump right breast	10/5/2009		No
60047	bruised left & right knee from fall	1/1/2010	1/5/2010	No
60047	chest/head cold	3/16/2010	4/18/2010	No
60048	pain in left hip	9/1/2009		No
60048	light headed ness	11/1/2009		No
60048	sore left ankle from fall	11/4/2009	11/15/2009	No
60049	allergies	8/21/2009		No
60050	vaginal bleeding	5/1/2009	11/1/2009	No
60050	constipation	11/1/2009	5/1/2010	No
60050	hysterectomy	11/9/2009	11/9/2009	No
60050	celiac disease	4/7/2010		No
60052	flu/upper respiratory infection	10/1/2009	10/1/2009	No
60052	UTI	3/14/2010	3/23/2010	No
60052	hot flashes	5/1/2010		No
60052	sinus infection	5/1/2010	5/14/2010	No
60053	pneumonia -bronchitis	6/10/2009		No
60055	bronchitis	9/28/2009	10/2/2009	No
60056	upset stomach	5/31/2009	6/9/2009	No
60056	constipation/bloating	5/31/2009	9/22/2009	No
60056	sinus infection	6/1/2009	7/1/2009	No
60056	head cold	2/16/2010	2/23/2010	No
60057	left foot stress fracture	5/27/2010		No
60058	left ankle sprain	4/17/2010	4/27/2010	No
60059	pre-diabetes	9/15/2009		No
60059	swollen ankle from fall	10/3/2009	10/10/2009	No
60059	dizzy passed out	11/20/2009	11/20/2009	No
60059	broken ft from fall	11/24/2009	1/3/2010	No
60059	diabetes	7/20/2010		No
60201	cold	4/16/2008		No
60207	stuffy nose	11/29/2008	12/5/2008	No
60208	high blood sugars	7/22/2008		No
60208	internal bruising/bleeding (due to auto accident)	7/22/2008	7/25/2008	Yes
60208	flu	11/10/2008	12/1/2008	No
60208	flu	2/10/2009	2/24/2009	No
60208	food poisoning	3/1/2009	3/1/2009	No
60208	upper resp infection	3/1/2009	3/1/2009	No
60210	skin fungus	2/9/2009	3/1/2009	No

60213	hematoma -left arm	6/6/2008		No
60214	dizziness with fast movements	7/17/2008	10/1/2008	No
60214	head cold	9/14/2008	9/25/2008	No
60214	right hip pain	2/1/2009	4/1/2009	No
60214	upper resp cold	2/24/2009	3/10/2009	No
60215	inflammation	8/4/2008		No
60218	abscessed tooth	7/10/2008		No
60218	pulled muscles -left chest	12/7/2008	12/12/2008	No
60218	pulled muscles in chest	3/1/2009	4/1/2009	No
60218	constipation	3/11/2009	3/12/2009	No
60218	head & chest cold	5/28/2009	6/15/2009	No
60219	bladder infection	8/15/2008	9/20/2008	No
60219	shingles	9/9/2008	9/20/2008	No
60219	sinus infection	10/22/2008	11/5/2008	No
60220	bronchitis	7/28/2009	8/3/2009	No
60220	flu	7/28/2009	8/3/2009	No
60222	head cold	10/11/2008		No
60222	back pain from car accident	1/16/2009		No
60223	blood in bowel movement	4/1/2009		No
60223	colitis	6/30/2009		No
60224	uterine fibroid	11/30/2008		No
60224	abnormal bleeding -menses	12/1/2008	12/1/2008	No
60224	uterine fibroid	12/1/2008		No
60227	sinus surgery	4/10/2009	4/10/2009	No
60228	left eye, left hip, left arm, and left ankle bruised	7/13/2009	7/13/2009	No
60228	flu	10/2/2009	10/9/2009	No
60228	left hip and buttocks bruised	1/4/2010	1/20/2010	No
60230	sinus infection	4/20/2009		No
60230	burning of throat	8/1/2009		No
60231	sinus infection	10/19/2009	10/30/2009	No
60233	sunburn face, neck arms	5/9/2009	5/14/2009	No
60234	back pain	10/1/2009		No
60234	respiratory restriction	2/18/2010		No
60237	sinus infection	3/1/2009	4/28/2009	No
60237	hemorrhoid	10/19/2009	10/23/2009	No
60241	scraped left arm	8/26/2009		No
60242	sinus stuffiness	2/2/2010		No
60244	irregular bowel movements/constipation	2/26/2009		No
60246	sinus infection	2/18/2009	3/3/2009	No
60246	displaced tailbone	5/29/2009	7/24/2009	No
60247	lower back pain/cramping	2/24/2009	3/20/2009	No

60247	urinary tract infection	6/16/2009	6/22/2009	No
60247	right elbow sore	12/1/2009		No
60247	sinus infection	12/18/2009	1/6/2010	No
60249	dull ache in left hip near groin when running	4/13/2009		No
60249	hurt back while running	7/16/2009	8/22/2009	No
60250	sore right hip from fall	3/4/2010	3/5/2010	No
60252	bruises on right hip from fall	9/3/2009	9/5/2009	No
60252	sinus infection	10/16/2009	10/26/2009	No
60252	shingles	11/16/2009	12/5/2009	No
60252	chest cold w/ cough & drainage	2/23/2010		No
60252	migraine	3/8/2010	3/8/2010	No
60253	back pain	2/1/2010		No
60255	sprained ankle	6/1/2009	7/1/2009	No
60256	fall-missed step scraped right knee	6/4/2009	6/4/2009	No
60259	upper respiratory infection/sinus infection	5/2/2009		No
60259	sores/pimples on head	7/1/2009		No
60259	back pain/shooting pain down right leg & backside	7/30/2009		No
60259	lipomas on left hip & right lower thigh removed	7/30/2009	7/30/2009	No
60259	plantar fascia release of left foot	7/30/2009	7/30/2009	No
60259	cold	8/31/2009	9/1/2009	No
60259	herniated disc	10/2/2009	10/29/2009	No
60259	back surgery to redo herniated disc	10/29/2009	10/29/2009	No
60259	numbness in right foot	10/29/2009		No
60259	worsening of sores/pimples on head	11/1/2009		No
60260	bronchitis	1/1/2010	1/25/2010	No
60261	cold/upper respiratory infection	9/15/2009	9/22/2009	No
60263	blister on right heel	8/1/2009	9/1/2009	No
60263	heavy bleeding and clotting	1/10/2010	6/1/2010	No
60267	sprained left ankle	7/14/2009	7/28/2009	No
60267	hot flashes	11/14/2009	11/15/2009	No
60267	missed/irregular menstrual cycle	12/1/2009		No
60268	sinus infection	6/13/2009	6/26/2009	No
60270	stomach virus	2/26/2010		No
60270	subarachnoid hemorrhage	3/10/2010		Yes
60270	frontotemporal craniometry/resection of basal gangli	3/17/2010	3/17/2010	Yes
60271	sinus infection	5/29/2009	6/5/2009	No
60271	sinus infection	10/5/2009	10/30/2009	No
60271	sinus infection	12/18/2009	1/4/2010	No
60271	sinus infection	1/7/2010	1/15/2010	No
60271	strep throat	2/9/2010	2/20/2010	No
60271	strep throat	3/6/2010	3/16/2010	No

60271	strep throat	4/23/2010	5/5/2010	No
60271	tonsillectomy with adnoidectomy	5/6/2010	5/6/2010	No
60271	pain from tonsillectomy	5/6/2010	5/12/2010	No
60401	allergy symptoms	3/20/2008		No
60401	right knee - torn medial meniscus	8/28/2008	1/29/2009	No
60402	sleepy	5/1/2008		No
60402	head/chest cold , sinus infection	1/15/2009		No
60404	lump on back of left leg	11/10/2008		No
60404	head cold	2/1/2009	2/1/2009	No
60407	mild gas	7/1/2008		No
60409	GI distress, shortness of breath, constipation , GERD	8/11/2008		No
60410	constipation	8/4/2008	9/9/2008	No
60411	gas & constipation	10/15/2008		No
60414	migraine	7/22/2009	7/23/2009	No
60415	bulging disc in 5th &6th vertebrae	2/26/2009		No
60421	back muscle pain	6/10/2009	6/18/2009	No
60422	upper respiratory tract infection	1/6/2010	1/14/2010	No
60426	worsened arthritis pain in hand	8/1/2009	12/1/2009	No
60426	gas	10/12/2009		No
60427	sore right ankle	7/5/2009		No
60428	food poisoning	7/16/2009	7/19/2009	No
60428	cold	10/23/2009	10/27/2009	No
60428	buttocks sore from fall	12/30/2009	12/30/2009	No
60429	pneumonia	8/9/2009	8/30/2009	No
60429	wisdom teeth removed	8/19/2009	8/19/2009	No
60429	flu	10/9/2009	10/18/2009	No
60429	motor sound in ear	2/1/2010		No
60431	tingling in fingers	5/8/2009		No
60432	bladder infection	5/22/2009	5/29/2009	No
60434	cold/sinus infection	11/8/2009	12/15/2009	No
60435	head cold	8/27/2009	9/4/2009	No
60435	heavy menstrual bleeding	10/1/2009	4/1/2010	No
60435	car accident - pinched nerve left arm	1/7/2010	3/1/2010	No
60435	sinus infection	2/7/2010	2/17/2010	No
60435	uterine ablation	4/7/2010	4/7/2010	No
60439	upset stomach "like something stuck in intestines"	3/10/2010		No
60442	nausea and vomitting	5/3/2010		No
60442	left foot pain	6/1/2010		No
60604	gas-bloating	8/15/2008	9/28/2008	No
60604	pregnant	3/1/2009		No
60606	gas/bloated (patient quit taking calcium)	1/7/2009	1/15/2009	No

60606	cold	9/24/2009		No
60610	cold, sore throat, cough, fever	11/29/2009	12/1/2009	No
60612	swelling in left foot & ankle	12/8/2009		No
60613	left ankle sore	6/10/2009	6/17/2009	No
60613	swollen right hand from hitting on a pole	9/1/2009	11/1/2009	No
60613	pain in left arm between elbow & shoulder	1/1/2010	3/1/2010	No
60615	frozen shoulder muscle strain	2/1/2010		No
60615	knee pain from fall (pain mainly when patient kneels)	3/5/2010		No
60617	positive TB skin test	6/4/2009		No
60617	joint stiffness	9/1/2009	10/1/2009	No
60617	sleepiness	9/1/2009	10/1/2009	No
60617	stiff neck/neck strain	11/2/2009	11/16/2009	No
60617	cold/ head congestion	3/20/2010	3/22/2010	No
60617	cavity/tooth decay	6/1/2010		No
60622	heart flutters	11/26/2009	11/29/2009	No
60625	hysterectomy with left salpingo-oopherectomy	3/16/2010	3/17/2010	No
60626	fainted post-blood donation	3/5/2010	3/5/2010	No
60629	bunion surgery	7/6/2010		No
60630	low iron	1/1/2010		No
60630	head cold	1/5/2010	2/1/2010	No
60630	sinus infection	1/15/2010	2/1/2010	No
60630	head cold	2/1/2010	2/1/2010	No
60630	head cold	3/1/2010	3/1/2010	No
60630	tired	6/1/2010		No
60630	loose stool	6/1/2010		No
60632	Urethra infection	10/1/2010	10/10/2010	No
60632	Bladder infection	10/1/2010	10/10/2010	
60632	Upper Respiratory Tract Infection	11/4/2010	11/4/2010	No
60634	Maxillary Hypoplasia Surgery	4/21/2011		Yes
60636	Gas Cramping	9/1/2010	9/1/2010	No
60636	Bruise on R leg from fall	3/14/2011	6/1/2011	No
60636	Stiff R leg from fall	3/14/2011		No
60637	Broken R ankle + Tibia	2/2/2011		Yes
60637	Common Cold	6/1/2011		No
60638	Scraped finger on L hand from fall	11/4/2010	11/4/2010	No
60638	Scraped Top of L Foot from fall	11/4/2010	11/4/2010	No
60638	Swollen L ankle	5/1/2011	7/1/2011	No

ADDENDUM

'The effect of vitamin D supplementation on calcium absorption in young women' will be presented as a poster at the Annual meeting of the American Society Bone and Mineral Research in October 2013. A paper is nearing completion. Jindal PS, Gallagher JC, Smith LM.Vitamin D supplementation on calcium absorption in Young women.

Tella SH, Jindal PS, Gallagher JC Skin color and serum 25OHD level: A comparison of young Caucasian and American American women. A poster at the annual meeting of the American Society Bone and Mineral Research in October 2013.

Glossary

DRI Dietary Reference Intakes
RDA Recommended Dietary Intake
IOM Institute of Medicine
HHS Department of Health and Human Services
USDA U.S. Department of Agriculture
WIC Women Infants and Children.
DOD Department of Defense
EAR Estimated Average Requirement
TUL Tolerable Upper Level (TUL)
WHO World Health Organization
25OHD serum 25-hydroxyvitamin D
NHANES NHANES - National Health and Nutrition Examination Survey ...
IRB Institutional Research Board
HRPO Human Research Protection Official
UNMC University Nebraska Medical Center
PTH Parathyroid hormone
AD/L is the percent of radioactive calcium (^{45}Ca) dose in 100mg calcium that is absorbed per litre of serum
NIH National Institutes Health